DISSERTATION

MENSTRUAL CYCLE CHARACTERISTICS IN WOMEN EXPOSED TO ATRAZINE IN DRINKING WATER

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY LORI ANN CRAGIN ENTITLED MENSTRUAL CYCLE CHARACTERISTICS AND REPRODUCTIVE PATTERNS IN WOMEN EXPOSED TO ATRAZINE IN DRINKING WATER BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

MENSTRUAL CYCLE CHARACTERISTICS IN WOMEN

EXPOSED TO ATRAZINE IN DRINKING WATER

Introduction

Atrazine is the most commonly used herbicide in the United States and a wide-spread groundwater contaminant. Concern regarding potential health effects of human exposure to atrazine is based on its well recognized designation as an endocrine disruptor. Studies have shown that menstrual cycle characteristics are markers for reproductive conditions. The specific hypothesis tested in this research was: Exposure to atrazine in municipal drinking water is associated with menstrual cycle abnormalities which, in turn, are modulated through a diminution of the pre-ovulatory luteinizing hormone surge. In addition, the following secondary hypothesis was tested: There is agreement between retrospective menstrual cycle questionnaire data and data obtained prospectively from menstrual cycle diaries. This study was the first to examine the effects of drinking water exposure to atrazine on menstrual function in humans and the first to examine the underlying mechanism of this association.

Methods

The state of Illinois was selected as the exposed study site location because of its intensive atrazine use. In 2005, 87% of Illinois corn was treated with 0.38 pounds of active ingredient applied per acre. The state of Vermont was selected as the comparison

site because of its low atrazine use. In 2005, 0.009 pounds of active ingredient per acre were applied. The study population was comprised of women 18 to 40 years old residing in Illinois and Vermont. Women participated by either answering a retrospective questionnaire describing their menstrual cycle characteristics, maintaining a prospective menstrual cycle diary and/or collecting daily urine samples through two menstrual periods. Participants provided two first morning urine voids (one on each day) and a total of four home tap water samples (two on each day) were collected for analyses of atrazine and atrazine degradation by-products. Participants also collected urine voids daily through two or more menstrual bleeding periods for determination of reproductive hormone levels and phase length. Results of municipal plant analyses (Syngenta Crop Protection, Inc. 2005) were obtained from the Illinois Environmental Protection Agency (EPA).

Crude and multivariable unconditional logistic regressions were used to assess the relationship between atrazine exposure and the menstrual cycle characteristics. Differences in means and both crude and multivariable linear regression were used to evaluate the relationship between drinking water exposure to atrazine (or markers of atrazine exposure) and menstrual cycle length as reported by the prospective menstrual cycle diary. Differences in means and crude and multivariable linear regression were also used to evaluate the potential relationship between drinking water exposure to atrazine (or markers of atrazine exposure) and the urinary concentrations of reproductive hormones including luteinizing hormone, pregnanediol 3-glucuronide, and estrone 3-glucuronide levels. Percent agreement, Cohen's kappa and the prevalence index were

used to compare retrospectively reported menstrual cycle characteristics with prospectively maintained menstrual cycle diary data.

Results

One hundred and two women participated in the study by answering a retrospective questionnaire (53 Illinois women and 49 Vermont women). Sixty seven of these women (65.7%) also maintained menstrual cycle diaries (30 Illinois women and 37 Vermont women). Thirty nine of these 102 women (38.2%) also provided daily urine samples for hormone analyses (18 Illinois women and 21 Vermont women).

Overall, levels of atrazine and atrazine metabolites were low in 2005, the year of data collection for this study, relative to previous and subsequent years. Atrazine levels in tap water were higher among Illinois women compared to Vermont women (p-value (p) = < 0.001). According to municipal plant monitoring (Syngenta Crop Protection, Inc.), atrazine averaged 0.21 parts per billion (ppb) in Mount Olive, Illinois and 0.29 ppb in Gillespie, Illinois in 2005.

Menstrual cycle length irregularity was associated with atrazine exposure, as estimated in several ways. Using state of residence as an exposure marker, women living in Illinois were more likely to report irregular menstrual cycle lengths (OR = 4.69; 95% CI: 1.58 – 13.95). In addition, a significant association, although imprecise, was observed between cycle length irregularity and residing more than four years in current home in Illinois (OR = 6.88; 95% CI: 2.08 – 22.78). As the number of years in the current home increased

among Illinois women, increasing odds ratios (ORs) were observed in a dose response manner. Although uncertainty exists because of the wide confidence intervals, a possible dose response association was also observed between the amount of unfiltered water consumed and menstrual length irregularity among Illinois women ($\leq$ 2 cups OR = 4.10, 95% CI: 1.24 – 13.51; > 2 cups OR = 5.73, 95% CI: 1.58 – 20.77 with Vermont women the comparison group).

Going more than six weeks without a menstrual period was significantly associated with residence in Illinois (OR = 6.16; 95% CI: 1.29 – 29.38). Elevated odds ratios, although not in a dose response manner, were also observed for years in current home and going more than six weeks without a menstrual period ($\leq$ 4 years OR = 9.68, 95% CI: 1.83 – 51.22; > 4 years OR = 3.76, 95% CI: 0.64 – 21.97 with VT women living in Vermont the comparison group).

There were no statistically significant associations between atrazine exposure and menstrual cycle length as measured with menstrual diary data. Since the latency period from atrazine exposure to menstrual cycle disruption is unknown, it is uncertain whether data collected from prospective diaries would reflect the present exposure or exposure months earlier.

A statistically significant association was present between municipal plant chlorotriazine monitoring data and follicular phase length in the adjusted linear model (β = -0.019; 95% CI: -0.04 – 0.00). When municipal plant data were used to calculate estimated 'dose'

(atrazine concentration x volume of unfiltered water ingested per day), a statistically significant increase in follicular phase length was observed with atrazine estimated 'dose' (β = -0.021; 95% CI: -0.04 – 0.00) and with chlorotriazine estimated 'dose' (β = -0.023; 95% CI: -0.04 – 0.00). These associations remained significant in the adjusted model.

Increased estimated 'dose' of atrazine and chlorotriazine were associated with decreased mid-luteal phase estrone 3-glucuronide levels for several markers. When municipal plant data were used to calculate estimated 'dose', mean mid-luteal phase estrone 3-glucuronide levels decreased but not significantly (atrazine: 29.45 ng/mg Cr compared to 20.62 ng/mg Cr, p = 0.36; chlorotriazine: 32.99 ng/mg Cr compared to 20.94 ng/mg Cr, p = 0.09). Using CDC (Centers for Disease Control and Prevention) analyzed samples to estimate 'dose', mid-luteal phase estrone 3-glucuronide levels were significantly decreased (both atrazine and chlorotriazine: 35.67 ng/mg Cr compared to 24.39 ng/mg Cr, p = 0.01). For the linear regression analysis, state of residence was imprecisely associated with mid-luteal phase estrone 3-glucuronide (β = -0.32; 95%CI: -0.68 – 0.04). Estrone 3-glucuronide was also imprecisely associated with atrazine exposure as determined by concentrations in residential tap water (β = -0.32; 95%CI: -0.68 – 0.04). When the amount of tap water consumed was taken into consideration to estimate 'dose', this association became stronger and statistically significant (β = -0.46; 95%CI: -0.82 – -0.10).

Exposure to atrazine through drinking water also appeared to have an effect on the concentration of progesterone during the luteal phase. Mean mid-luteal phase

Exposure to atrazine through drinking water also appeared to have an effect on the concentration of progesterone during the luteal phase. Mean mid-luteal phase pregnanediol 3-glucuronide levels decreased with increasing atrazine (7.92 μg/mg Cr compared to 12.44 μg/mg Cr, p = 0.02). Results of the linear regression analysis were in agreement as mid-luteal phase pregnanediol 3-glucuronide was statistically significantly associated with atrazine estimated 'dose' when using municipal plant data (β = -0.57; 95% CI: -1.06 – -0.09).

Although not statistically significant, atrazine exposure appeared to be associated with small but consistent reductions in preovulatory luteinizing hormone concentrations across the various atrazine exposure variables.

For the retrospective versus prospective menstrual cycle data analysis, a regular menstrual cycle was defined two different ways (definition 1 = 25-30 days; definition 2 = 25-35 days). A high overall agreement was observed between retrospective questionnaires and prospective diaries (69% and 75% for definitions 1 and 2, respectively) but unadjusted Cohen's kappas were low (0.31 and 0.43 for definitions 1 and 2, respectively). The prevalence indices were -0.33 for definition 1 and -0.66 for definition 2.

Conclusions

Although the majority of atrazine concentrations in municipal drinking water measured in this study were below the EPA standard for drinking water (3.0 ppb), exposure to atrazine

was associated with altered menstrual cycles. Menstrual cycle length irregularity, increased follicular phase length and increased cycle length were significantly associated with atrazine exposure. Moreover, the reproductive hormone results provided further support of the menstrual cycle findings and offer the possibility of reduced fecundability in women exposed to atrazine. Given the dependence of reproductive hormones on one another, any hormone modifications could lead to a menstrual cycle alteration.

In addition, there was agreement between data for menstrual cycle characteristics reported retrospectively from questionnaires and data obtained prospectively from menstrual cycle diaries. Therefore, although Cohen's kappa was low, it was shown to have been kept deceptively low by the high likelihood of chance agreement resulting from the high prevalence effect. The demonstration of the effect of prevalence on the kappa statistic was a major finding of this study. The major strengths of the study were availability of tap water samples, urinary analyses of atrazine, urinary concentrations of reproductive hormones and both retrospective as well as prospective measurement of menstrual cycle activity. The major limitations were the relatively low levels of atrazine measured in Illinois drinking water during 2005 and the small number of subjects. Further studies, on larger populations, are needed to confirm the findings of this study.

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Dedication

To my dear mother and grandmother

Two women who sacrificed so I had the opportunities they did not.

Thank you for believing in me even when I did not believe in myself. Your unwavering acceptance and love has given me the confidence to accomplish all that I have.
I love you.

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And finally, I thank Shayne for his love and support throughout the many years of this research, for putting up with me during the challenging times and for always taking a genuine interest in what I do.

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1. INTRODUCTION, HYPOTHESIS AND SPECIFIC AIMS

Introduction

This study was the first to examine the effects of exposure to atrazine in drinking water on menstrual function in humans. To the best of my knowledge, it was also the first to examine the underlying mechanism of this possible association. In 2002, 7.4 percent or approximately 2.1 million married couples were reportedly infertile (1). Of the 61.6 million women of reproductive age in 2002, 7.3 million had used an infertility service in an attempt to either become pregnant or prevent miscarriage (1). Studies have shown that menstrual cycle characteristics are markers for reproductive conditions such as such as infertility (2), fecundability (3), breast cancer (4, 5, 6, 7, 8, 9, 10, 11), ovarian cancer (12), ovarian cysts (13), uterine fibroids (14), spontaneous abortions (2), and endometriosis (13, 15) as well as conditions like diabetes (16), osteoporosis (17), cardiovascular disease (18, 19), and chronic diffuse pain (20). In addition, changes in menstrual cycle length have been associated with exposure to persistent organochlorine compounds (POCs) (21) including polychlorinated biphenyls (PCBs) (22, 23) and dioxin (24), pesticides (25) including dichlorodiphenyl trichloroethene (DDT) (26, 27, 28), organic solvents (29) including benzene (30), diethylstilbestrol (DES) (31), soy milk (32), chlorination by-products(33), caffeine (34), cold temperatures (35), and certain medications (36, 37, 38, 39). Certain factors such as work shift (40), stress (41, 42), fat intake (43, 44), fiber intake (44), smoking (43, 45), age (43, 46), disordered eating (47), schedule variability (35), depression (45), alcohol consumption (46, 48), body mass index (BMI) (43, 45, 49), social economic status (49), and physical activity (48, 50) have also

been associated with changes in cycle length. Menstrual cycle patterns result from biological systems that depend upon a woman's hormonal status and that are sensitive to environmental influences (45). Environmental influences that disrupt menstruation should be considered capable of disrupting normal reproductive function (45).

Despite the ubiquitous use of pesticides, comparatively little is known about their adverse health effects, particularly reproductive effects, in humans (51). Reproductive function can be compromised by exposure to endocrine disrupting chemicals (52). Many pesticides may disrupt reproductive or endocrine function in animals and humans; however, for several pesticides there is little or no information available on the potential for endocrine disruption (52) and even less is known about the underlying biological mechanism. The goal of this research was to explore the relationship between atrazine, a known endocrine disruptor, and reproductive health by examining menstrual cycle characteristics among premenopausal women.

Atrazine is the most commonly used herbicide in the United States and a wide-spread groundwater contaminant (53). Concern regarding potential health effects of human exposure to atrazine is based on its well recognized designation as an endocrine disruptor. In laboratory studies, atrazine induces estrogenic effects in frogs such as hermaphroditic deformities and demasculinization at concentrations 30 times lower than the current Environmental Protection Agency (EPA) drinking water standard of 3.0 parts per billion (ppb) (54). The endocrine disrupting properties of atrazine appear to be due to its effect on the hypothalamic-pituitary-ovarian axis and its ability to promote the conversion of

testosterone to estrogen through activity of the enzyme aromatase. The endocrine disrupting effects of atrazine have resulted in elevated estrogen levels and retarded gonadal development in amphibians (53, 55), decreased androgens and androgen inhibition in male rats (56, 57), altered cyclicity in female rats (58, 59), pregnancy loss in rats (60), decreased uterine and ovarian weights in rats (61) and the induction of aromatase, the rate-limiting enzyme in the conversion of androgens to estrogens in a human cell line (62). The biological properties of atrazine demonstrated in animal models suggest human exposure could result in impaired fertility, adverse reproductive outcomes and an increased risk of hormonally dependent cancers.

Hypothesis

Menstrual cycle characteristics have implications for women's fecundity and risk of hormonally related diseases (25, 63). Altered menstrual cycle characteristics have been associated with disturbances of the luteinizing hormone (LH), an anterior pituitary gonadotrophin. Since LH is essential for normal menstrual function, a reduction in the LH surge could affect the ovarian cycle and lead to an altered menstrual cycle.

Therefore, the specific hypothesis tested in this research was: **Exposure to atrazine in municipal drinking water is associated with altered menstrual cycles. These alterations are modulated through a diminution of the pre-ovulatory LH surge.**

In addition, the following secondary hypothesis was tested: **There is agreement between data for menstrual cycle characteristics reported retrospectively from questionnaires and data obtained prospectively from menstrual cycle diaries.**

Specific Aims

The specific aims of the study were to:

1. Recruit 100 women from Mount Olive and Gillespie, Illinois and Waterbury and Fair Haven, Vermont to participate in the study.

2. Describe the characteristics of the study population; specifically, the demographic characteristics, potential confounders and effect modifiers.

3. Characterize atrazine exposures in water among participating women living in the Illinois and Vermont study populations. Specifically, characterize markers of atrazine exposure (state of residence, years in current home, and consumption of unfiltered water) and atrazine exposure (residential water samples analyzed with and without chlorine; chlorotriazine residential water samples analyzed with and without chlorine; urinary desethylatrazine mercapturate (DEAM); atrazine municipal plant monitoring (Syngenta Crop Protection, Inc. 2005); chlorotriazine municipal plant monitoring (Syngenta Crop Protection, Inc. 2005); atrazine estimated 'dose' using municipal plant concentrations; chlorotriazine estimated 'dose' using municipal plant concentrations; atrazine estimated 'dose' using

Centers for Disease Control and Prevention (CDC) atrazine concentrations; chlorotriazine estimated 'dose' using CDC chlorotriazine concentrations.

4. Characterize menstrual cycle characteristics among participating women living in the Illinois and Vermont study populations. Specific characteristics include menstrual cycle length regularity, length, spotting between menstrual periods, going more than six weeks without a menstrual period, and dysmenorrhea.

5. Characterize reproductive hormones and the follicular and luteal phase lengths among participating women living in the Illinois and Vermont study populations. Specific reproductive hormones include preovulatory and peak LH, mid-luteal phase estrone 3-glucuronide ($E_1$3G), and follicular and mid-luteal phase pregnanediol 3-glucuronide (Pd3G).

6. Evaluate the relationship between menstrual cycle characteristics as reported by the retrospective questionnaire and markers of atrazine drinking water exposure.

7. Evaluate the relationship between menstrual cycle length as reported by the prospective menstrual cycle diary and atrazine drinking water exposures (or markers of atrazine drinking water exposure).

8. Evaluate the relationship between levels of reproductive hormones associated with infertile ovulatory cycles measured in urine and atrazine drinking water

exposures (or markers of atrazine drinking water exposure). Further, evaluate the LH peak as well as follicular phase length with respect to atrazine exposure.

9. Evaluate the already established relationship between phase lengths (luteal and follicular) as well as peak Pd3G and menstrual cycle characteristics. The menstrual cycle characteristics to be evaluated include menstrual cycle length and length regularity (regular vs. irregular), and interval without a menstrual period (> six weeks vs. $\leq$ six weeks).

10. Compare retrospectively reported menstrual cycle characteristics with prospectively maintained menstrual cycle diaries.

2. Literature Review

Use and Health Effects of Pesticides

For more than 60 years, large amounts of xenobiotics have entered the environment through efforts to increase agricultural productivity (64). In 2001, world pesticide use surpassed 5.0 billion pounds and use in the US exceeded 1.2 billion pounds (65). The agricultural sector accounts for more than 75% of pesticide use. Some pesticides are suspected endocrine disruptors, capable of interfering with the production, release, transport, metabolism, binding, action or elimination of hormones responsible for the maintenance of homeostasis and the regulation of developmental processes (66). This interference can result in adverse health consequences such as increased cancer incidence and reduced reproductive function (52). Exposure to pesticides can lead to reproductive dysfunction through one of the following mechanisms: direct damage to the structure of the cell, interference with biochemical processes necessary for normal cell function or biotransformation resulting in toxic metabolites (67).

Although the majority of studies of health and exposure to pesticides have focused on males, studies examining the effects of pesticide exposure on female reproductive health have been reported (25, 51, 68, 69, 70, 71, 72, 73, 74, 75, 76). Fuortes et al. (73) evaluated infertility among women with an agricultural related work history and found an increased risk of medically confirmed infertility (Odds Ratio (OR) = 7.0, 95% Confidence Interval (CI): 2.3 – 20.8). Smith et al. (76) reported an increase in medically diagnosed infertility in women exposed to pesticides (OR = 3.82; 95% CI: 1.28 – 11.42).

A cross-sectional study of first pregnancies among Colombian women working in flower production, where a range of pesticides are used, found an OR for fecundability (FR) of 0.73 (95% CI: 0.63 – 0.84) for 2 years or more of work (74). A 2000 Danish study also suggested a risk of reduced fecundability among female greenhouse workers, especially among workers not using gloves (FR = 0.67, 95% CI: 0.46 – 0.98) (71). A matched record-based case-control study of neural tube defects conducted in Mexico suggested that children of mothers who worked in agriculture have a greater risk of anencephaly (OR = 4.57, 95% CI: 1.05 – 19.96) when pesticide exposure occurred three months before to one month after the mother's last menstrual period (75). Bell et al. (69) reported slightly elevated risks of fetal death among women who, during the second trimester, were living within one mile of the application of carbamates or estrogenic pesticides (hazards ratio (HR) = 1.3, 95% CI: 1.0 – 1.8 and HR: 1.4, 95% CI: 0.8 – 2.5, respectively). Shaw et al. (70) also examined proximity to an agricultural crop and reproductive health. They reported women living within 0.25 miles of an agricultural crop were at an increased risk for offspring with neural tube defects (OR = 1.5, 95% CI: 1.1 – 2.1) (70). Birth defects were also studied in a nationwide Finnish case-control study of 2,612 infants where an excess of oralfacial clefts among children of mothers in agricultural work during their first trimester of pregnancy was observed (OR = 1.9, 95% CI: 1.1 – 3.5) (77). When all birth defects were pooled and agricultural work was compared with nonagricultural work in the first trimester of pregnancy, the adjusted OR was 1.4 (CI = 0.9-2.0) (77). Eskenazi et al. (72) reported associations between in utero pesticide exposure and decreased gestational length among a cohort of Latina women living in an agricultural community in California.

Since pesticides have dissimilar chemical structures and toxicities, using the broad definition of 'pesticide' does not help to identify the etiologic pathway of their effects (78). Although classifying pesticides based on pesticide class or functional group is more specific, it is still limiting (25). Categorizing pesticides as hormonally active or as endocrine disruptors is more informative (25). Furthermore, information on the specific hormone potentially affected by endocrine disrupting pesticides and the anticipated direction of the disruption can be beneficial in revealing pesticide-reproductive health effect relationships (79). Classified as neither an agonist nor an antagonist, atrazine has been found to have a low affinity for androgen and estrogen receptors and not be directly estrogenic or anti-estrogenic (80, 81, 82). One study did report atrazine and diaminochlorotriazine bound to the estrogen receptor (83); however, an extremely high concentration of atrazine was required to displace the natural ligand which may have been the result of a denatured receptor and not actual competitive binding (84). Atrazine is an endocrine disruptor previously found to inhibit the LH surge as well as induce the enzyme aromatase in animals (56, 57, 83, 85, 86, 87, 88, 89).

Use and Presence of Atrazine

Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is a triazine herbicide first registered in 1958 to control broadleaf and grassy weeds. Similar to most herbicides, atrazine works by inhibiting photosynthesis. By taking the place of the electron carrier molecule quinone, atrazine disrupts the electron transport process and ultimately blocks photosynthesis. Although several European countries have banned

atrazine, approximately 32 million kilograms are applied annually in the United States to crops such as corn, sorghum and sugar cane (90), making it the most commonly used herbicide by weight in the United States (91). According the United States Geological Survey's (USGS) National Water-Quality Assessment Program, atrazine was the most commonly found pesticide in agricultural streams (90). Consistent with USGS findings, the EPA found atrazine to be the most commonly detected pesticide in surface water and the second most frequently detected pesticide in drinking water, with peak concentrations appearing in the spring when rainfall is high and atrazine applications are most frequent (92).

For such a widely used herbicide, found widespread in our drinking water supply, there is a clear need for accurate drinking water exposure assessment. According to Barr et al. (93), atrazine exposure in humans has been underestimated in the past. Previous reports have focused on atrazine mercapturate as the primary atrazine metabolite; however, new data demonstrate diaminochlorotriazine and desethylatrazine are the principal metabolites detected (93). Barr et al. (93) also report the principal metabolites present depend upon the exposure scenario. Since atrazine can be broken down in the environment by bacteria and abiotic processes, environmental exposures are typically dominated by the chlorinated atrazine metabolites which are considered equal in toxicity to the parent compound.

Epidemiologic Studies of Atrazine

Although inconclusive, epidemiologic investigations have suggested associations between atrazine and/or triazine exposure and adverse health outcomes including cancers, reproductive effects and chronic conditions such as diabetes. In a pooled analysis of three case-control studies with a combined sample size of 3,417, De Roos et al. (94) reported that the use of several pesticides, including atrazine, was significantly associated with an increased risk for non-Hodgkin's lymphoma. Individually, these three case control studies reported moderate associations between atrazine exposure and non-Hodgkin's lymphoma, although not all were statistically significant (95, 96, 97). Using data from the Agricultural Health Study, Rusiecki et al. (98) also observed suggestive trends in risk for non-Hodgkin's lymphoma, in addition to multiple myeloma, lung cancer and bladder cancer, among applicators of atrazine. Although the cohort was large (36,513 applicators) and employed a prospective study design, the average follow-up period was only 6.5 years and few cancers had occurred during this time period; therefore, statistical power was limited (98). However, it was the stated intention of the authors to continue to monitor the applicators as more cases of cancer develop. Agricultural Health Study data have also revealed an association between atrazine exposure and wheezing with a dose-response trend among those applying atrazine more than 20 days per year (OR = 1.5, 95% CI: 1.2 - 1.9) (99).

Atrazine in drinking water was associated ($p < 0.05$) with stomach cancer in an ecologic study in Ontario, Canada, during 1987 to 1991 (100). An ecologic study exploring spatial patterns of childhood cancers in Maryland found children exposed to atrazine,

nitrates and metolachlor were more likely to develop one of four types of cancer (brain, bone, leukemia, or lymphoma)(OR=7.6, 95% CI: 4.2 – 13.7) (101). Using data from the California cancer registry, Mills (102) reported associations between atrazine use and testicular cancer, leukemia and brain cancer among Hispanics and prostate cancer among blacks. It should be noted the findings were not statistically significant among Hispanics, and that individual exposure data were not available since the study was ecological in design (102). In an Italian case-control study, Donna et al. (103) reported an association between herbicide exposure and ovarian mesotheliomas, with a possible role suggested for triazine exposure. Exploring these findings further, Donna et al. (104) found a 2.7 times greater risk (95% CI: 1.0 – 6.9) of epithelial ovarian cancer among women with a previous exposure to triazines as compared to women not previously exposed (104). Although doses could not be quantified among the study subjects, trends in risk with duration of exposure and probability of exposure were noticeable in the data (104).

To date, reported associations between breast cancer in humans and atrazine exposure have varied in magnitude and direction. A study in Kentucky revealed a statistically significant increase in breast cancer risk in counties with medium and high levels of triazine exposure compared to counties with low levels (105). However, the increase was weak (OR=1.14, 95% CI: 1.08 – 1.19 for medium levels and OR= 1.2, 95% CI: 1.13 – 1.28 for high levels) and may be attributable to the study's ecological design since exposure was estimated based on corn crop production, pesticide use data and water contamination data instead of measurements in individuals (105). Using Kentucky breast cancer registry data and an ecological study design, Hopenhayn-Rich et al. (106) failed to

detect an association for breast cancer and atrazine exposure when atrazine exposure was derived from public water measurements, acres of corn planted and pounds of atrazine sold. A California study of Hispanic women found that the risk of breast cancer was not significantly associated with use of the triazine herbicides atrazine or simazine (107). This study was also ecological and assumed county level measures of pesticide use were relevant to individuals (107). In yet another ecological study, the findings of Muir et al. (108) suggested an association between breast cancer incidence and atrazine application. Recently, McElroy et al. (109) reported that exposure to atrazine in drinking water (1.0-2.9 ppb) was not associated with an increased risk of breast cancer (OR=1.1, 95% CI: 0.9 - 1.4). Due to the small number of samples above the EPA maximum contaminant level (MCL) (<4%), their results were inconclusive for concentrations at or above the EPA drinking water standard of 3.0 ppb (109).

Several epidemiological studies have observed a relationship between triazine exposure and prostate cancer (102, 110, 111, 112). Mills and Yang (111) conducted a nested case-control study drawing predominantly on Hispanic farm workers in the labor union and concluded that high levels of exposure to triazine herbicides elevate risk of prostate cancer when compared to workers with lower levels of triazine exposure. Additionally, a series of studies have been conducted among workers at a Louisiana atrazine manufacturing plant that opened in 1970. Workers at the plant had about twice the prostate cancer incidence when compared to the regional general population (112). However, the study was based on relatively small numbers of cases and suffered from potential detection bias due to the introduction of prostate-specific antigen (PSA)

screening in the worker cohort during the period of observation. MacLennan et al. (113) also looked at mortality in the same population of plant workers and found employees had a greater than expected number of non-Hodgkin's lymphoma deaths even with limitations of a small sample size and short follow-up time (113). Among pesticide applicators from Iowa and North Carolina participating in the Agricultural Health Study, no association was found between atrazine exposure and prostate cancer, although a limitation of the study was a relatively short follow-up (114).

Although epidemiological studies on atrazine's potential reproductive effects in humans are limited in number, associations have been found between atrazine exposure and intrauterine growth retardation (IUGR), premature birth, small for gestational age, and spontaneous abortion (68, 115, 116, 117). In a study of more than 3,500 births, Villanueva et al. (116) found atrazine levels in municipal drinking water were associated with small for gestational age (OR = 1.5, 95% CI: 1.11 - 2.13) if the entire third trimester occurred during the period of the highest atrazine drinking water levels (May to September). An increased relative risk for IUGR was found in communities in Iowa with high levels of atrazine in drinking water as compared to communities with low atrazine levels (risk ratio (RR) = 1.8, 95% CI: 1.3 - 2.7) (115). However, the authors report their findings should be considered preliminary since the study was ecological in design and therefore potentially limited by confounding (115). Using data from the Agricultural Health Study, Saldana et al. (118) found women who reported ever having used atrazine during their first trimester of pregnancy were more likely to have gestational diabetes mellitus. Recently, a statistically significant positive correlation

between the incidence of abdominal wall defects and atrazine surface water levels was found in Indiana (119). Arbuckle et al. (68) observed an increased risk of spontaneous abortions for exposure to triazines prior to conception (OR=1.4, 95% CI: 1.0 – 2.0). More than 2,000 women participating in the Ontario Farm Family Health Study provided data on close to 4,000 pregnancies to show there was a critical window of exposure for spontaneous abortions (68). The critical window included the three months prior to conception through the month of conception. Similarly, Savitz et al. (117) found evidence of an association between male pesticide exposure three months before conception through the month of conception and adverse reproductive outcomes. Their findings suggested that mixing or applying herbicides was associated with preterm delivery (OR=2.1, 95% CI: 1.0 – 4.4). Moreover, mixing or applying atrazine in the yard was significantly associated with preterm delivery (OR=4.9, 95% CI: 1.6 – 15). Swan et al. (120) found that exposure to agricultural pesticides was associated with reduced semen quality. Atrazine levels were elevated in Missouri men with sperm concentrations below the median as compared to Missouri men with sperm concentrations above the median (p-value (p) = 0.01) (120). High levels of atrazine were associated with an increased risk of poor semen quality (OR = 11.3), although the CI was very wide (95% CI: 1.3 – 98.9) (120).

Toxicological Studies of Atrazine

The chlorinated triazines and their metabolites have been shown to cause adverse neuroendocrine, reproductive and immune effects in laboratory animal systems. Immune system impairment has been reported in rats, mice and amphibians dosed or exposed to

2.7 mg/kg, 250 mg/kg and 21 ppb, respectively (121, 122, 123). Exposure to 100 mg/kg of atrazine was associated with neurotoxicity in dopaminergic systems, systems important for cognition and movement (124). Consistent with these findings, erratic and hyperactive swimming behavior and changes in physiological capabilities have been observed in fish and salamanders dosed or exposed to 0.001 mg/L and 400 mg/L of atrazine, respectively (125, 126, 127).

Lifetime feeding of 400 ppm atrazine to Sprague-Dawley rats lengthened their estrous cycle, increased the number of days in estrus and resulted in an earlier onset of mammary tumors (55, 59). Atrazine has also been shown to delay the onset of puberty and alter estrous cyclicity in female Wistar rats dosed 200 mg/kg atrazine (58). In the F344 rat, feeding of 750 ppm of atrazine was associated with an increase in breast tumors in males (128).

In addition, exposure to 21.0 ppb of atrazine for as little as 48 hr resulted in severe gonadal dysgenesis in African clawed frogs (*Xenopus laevis*) (129). Atrazine induced hermaphroditism at concentrations of only 0.1 ppb when administered to frogs throughout larval (tadpole) development (54). Exposure at these low levels also resulted in retarded gonadal development and testicular oogenesis in leopard frogs (*Rana pipiens*) (53). Slower developing males even showed oocyte growth (53). Hayes et al. (53) have shown that increased rates of gonadal dysgenesis and hermaphroditism occur in the wild at atrazine-contaminated sites across the United States. The atrazine concentrations in many water sources in the United States exceed the effective concentrations used in these

laboratory experiments. Increased feminization has also been reported in fish exposed to 1.0 mg/L and rodents dosed 120 mg/kg (56, 57, 130). Recently, in a study of pesticide and nutrient inputs from areas of intensive row crop agriculture for corn and soybean production, McDaniel et al. (131) reported the higher the concentration of atrazine and nitrate and the higher the number of pesticide detections, the greater the proportion of testicular oocytes in frogs.

Pregnancy loss (i.e., litter resorption) and decreased litter size were seen in rats dosed 50 mg/kg atrazine (60). More specifically, pregnancy loss occurred when atrazine was fed during gestation days 6-10 (i.e., a time when sufficient withdrawal of LH may terminate a pregnancy), but not when it was fed during gestation days 11-15 (60). Pregnancy loss (and a decrease in body weight) was also reported to be associated with atrazine exposure in rats by Cummings et al. (132) Prostate inflammation was observed in male rat offspring when their mothers were given atrazine while nursing (133). Recently, Lenkowski et al. (134) examined exposure of frogs to high levels of atrazine (10-35 parts per million (ppm)) in a short duration (< 2 days) and found a significant dose-dependent relationship between atrazine exposure and organ development. The authors reported these malformations were the result of increased levels of cell death in the kidney and midbrain (134).

Luteinizing hormone, estrogen and progesterone

Atrazine appears to affect the central nervous system at the level of the hypothalamus (not the pituitary) producing a diminution of the ovulatory LH surge (48, 60, 83, 84, 86,

135, 136, 137). Cooper et al. (86) reported that atrazine suppresses LH and prolactin levels in Sprague-Dawley and Long-Evans hooded female rats by altering hypothalamic control. The LH and prolactin surges were suppressed after a single 300 mg/kg dose to Long-Evans hooded rats. Both hormones were suppressed in a dose-response manner in both Sprague-Dawley and Long-Evans hooded rats dosed at 75, 150 and 300 mg/kg/day for 21 days (86). McMullin et al. (83) reported atrazine and diaminochlorotriazine, one of atrazine's primary chlorinated metabolites, both decreased total plasma LH levels of Sprague-Dawley rats. Narotsky et al. (60) showed a LH mechanism of pregnancy loss in Sprague-Dawley female rats when atrazine was administered during a time when sufficient withdrawal of LH could terminate a pregnancy. LH effects were also reported in Sprague-Dawley rats by Trentacoste et al. (136) when 100 mg/kg/day of atrazine was administered by gavage for approximately 20 days. When dosed for three days at 200 mg/kg, Cooper et al. (135) noted almost complete inhibition of the LH surge in both Long Evans hooded and Sprague-Dawley rats.

In addition, toxicological studies have shown atrazine disrupts estrogen and progesterone. Inconsistencies in the effects of atrazine on estrogen have been reported with both elevations and reductions observed (59, 61, 130, 132, 138, 139, 140). Wetzel et al. (59) reported plasma estrogen concentrations more than doubled in Sprague-Dawley female rats fed 70 ppm atrazine for three months. Cummings et al. (132) also found estradiol was significantly increased when Sprague-Dawley female rats were dosed with 200 mg/kg atrazine prior to the prolactin surge. However, Eldridge et al. (61) reported a decrease in plasma estradiol in Sprague-Dawley female rats dosed with 100 and 300

mg/kg/day of atrazine. In goldfish exposed to atrazine for 21 days, Spano et al. (130) reported a suppression of testosterone and an elevation of estrogen in a time and dose related manner. In male African clawed frogs (Xenopus laevis) exposed to 1.0 μg/L atrazine in their water tank, Coady et al. (138) observed lower estradiol levels compared to controls, although the effects were not seen at other (i.e., both lower and higher) atrazine concentrations. In pigs fed atrazine, a decrease in estradiol was observed (139, 140).

Studies have shown effects by atrazine exposure on progesterone secretions, although both increased and decreased levels have been observed (132, 135). In Sprague-Dawley and Long-Evans Hooded rats fed 150 mg/kg/day of atrazine for four days, Cooper et al. (135) reported elevated serum progesterone and prolonged periods of diestrus. However, in Holtzman rats, a statistically significant decrease in serum progesterone and loss of implantation was observed at 100 and 200 mg/kg/day when dosed with atrazine on days 1-8 of pregnancy and necropsied on day 8 or 9 (132).

Mechanism of Action

Although the cellular mechanism of central nervous system disruption is largely unknown (84), a reduction in the LH surge has been attributed to a decrease in hypothalamic catecholaminergic dopamine level in rats (124, 141, 142) and mice (143). Recently, Hossain and Filipov (144) reported atrazine and its chlorinated atrazine metabolites, desisopropylatrazine and desethyl atrazine, but not diaminochlorotriazine, are responsible for the dopamine decrease. The inhibition of the LH surge results in

changes similar to those that occur during reproductive aging in rats (86). Since reproductive aging in rats and humans differs and the oestrus and menstrual cycle are not exactly the same, the applicability of rodent findings to humans remains in question. Reproductive senescence in humans is marked by menopause and is the result of a depletion of primary follicles. In rats, reproductive senescence typically occurs at one year of age, is the consequence of impaired hypothalamic function and results in constant estrus (84, 137). At the time of reproductive senescence (i.e., constant estrus in rats and menopause in humans), levels of estrogen and prolactin in the rat are greater than in the human where estrogen and prolactin is essentially negligible (84). Nevertheless, control of LH and prolactin is similar in the two species and, therefore, atrazine could disrupt the levels of these crucial pituitary hormones (84). In addition, since the hypothalamic-pituitary-ovarian axis relies on feedback loops, a change in any hormone level could affect levels of other reproductive hormones.

Toxicological findings of elevated estrogen support the proposed mechanism of action that atrazine induces the production of aromatase, which is part of the P450 family of enzymes responsible for converting androgens to estrogens (88). The induction of aromatase results in a depletion of testosterone and an increase in estrogen. This was originally discovered in human cell lines by Sanderson et al. (62) and has recently been explained further by Fan et al. (81). Atrazine increases aromatase by binding to phosphodiesterase and inhibiting its action, increases cyclic AMP (cyclic adenosine monophosphate) activity resulting in increased transcription of cytochrome 19 (CYP19) and, thereby, increases aromatase activity (81). In addition to binding phosphodiesterase,

Fan et al. (81) also showed atrazine binds to the steroidogenic factor 1 (SF-1) which also increases aromatase promoting activity, in that atrazine only affects aromatase expression in cells and tissues that use the SF-1 dependent aromatase promoter.

In sum, it is possible that atrazine works by more than one mechanism of action and the precise cellular mechanisms have not been fully understood. However, changes to the hormonal environment further support the hypothesis that atrazine exposure changes the neuroendocrine control and ultimately leads to altered menstrual cycle characteristics and possibly infertility (80, 84, 86).

The Menstrual Cycle

The menstrual cycle represents a complex interaction between the nervous, endocrine, and reproductive systems or, more specifically, the interaction primarily between the central nervous system (CNS) including the hypothalamus, anterior pituitary gland, ovaries and the endometrium (145, 146). The menstrual cycle culminates in the production and release of a mature ovum and an environment able to support fertilization and maintain pregnancy (146). It is a process nearly exclusive to humans and a few nonhuman primates (145).

The cycle is hormonally controlled by the hypothalamic-pituitary-ovarian axis. The first half of the menstrual cycle, from menses onset to ovulation, is the proliferative or follicular phase of the cycle (Figure 1.1). During this phase, the follicles grow, a dominate follicle emerges, and estradiol stimulates the growth of the endometrium from a

height of 1 mm to 5mm (145). The follicular phase is estrogen dominated with estradiol being the primary estrogen (145). The monthly interplay of hormones is driven by pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus to stimulate the pulsatile release of follicle stimulating hormone (FSH) and l uteinizing hormone (LH) (147). Responding to FSH, a dominant ovarian follicle matures, releasing increasing levels of estrogen, the result of the aromatization of androgens by the granulosa cells of the follicles (145). The rise in circulating estrogen levels eventually induce a surge release of LH and FSH. The LH surge induces ovulation which stimulates the release of the ovum from the follicle of the ovary. The period from ovulation to the next menses is the luteal or secretory phase (147). Ovulation occurs and the follicle, having released its ovum, transforms into the corpus luteum (147). The corpus luteum secretes increased amounts of estrogen and progesterone resulting in increased plasma estrogen and progesterone levels. Increased estrogen and progesterone levels cause a decrease in the secretion of the gonadotropins (FSH and LH) (148). If pregnancy does not occur, the corpus luteum regresses in approximately two weeks and the hormone levels of progesterone and estrogen go back down. With decreased estrogen and progesterone levels, the endometrium degenerates resulting menstrual bleeding results and a new surge of GnRH occurs, starting the cycle over again (147). If the egg is fertilized, the corpus luteum begins to produce the hormone human chorionic gonadotropin (hCG) (147). This hormone sustains the corpus luteum and, therefore, levels of estrogen and progesterone which prepare the endometrium for the implantation of the fertilized egg (147). Later in gestation, estrogen and progesterone are produced by the placenta. Disruption of this endocrine axis can result in compromised follicular

development, implantation, menstrual irregularities, and/or adverse reproductive outcomes (146).

Clinicians and epidemiologists suggest menstrual cycle patterns provide a view into female reproductive biology (45). For males, semen monitoring is a noninvasive technique capable of providing insight into their reproductive health; however, noninvasive sampling techniques to assess ova and the uterus do not exist for females. Since the menstrual cycle can be examined noninvasively and prospectively, menstrual cycle characteristics have proven to be important indicators of reproductive health (149). Rowland et al. (45) have suggested menstrual cycle patterns are influenced by a number of host and environmental characteristics and the factors that perturb menstrual cycle function may increase a woman's risk of other reproductive disorders. In a longitudinal study of 215 women with no known fertility problems, Baird et al. (150) found that menstrual cycles varied hormonally and predicted fertility. In their study, women with cycles with long follicular phases lengths, higher follicular phase progesterone, lower preovulatory LH, low midluteal phase progesterone and lower midluteal phase estrogen were less likely to conceive (150).

Menstrual cycle length data have been analyzed using methods for analysis of continuous (21, 23, 24, 27, 30, 32, 33, 34, 36, 39, 42, 50, 151, 152, 153, 154, 155, 156, 157) and categorical variables (2, 3, 5, 6, 9, 12, 13, 14, 16, 17, 18, 20, 23, 25, 26, 29, 30, 33, 34, 35, 38, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 153, 154, 155, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167) with the referent group often not the same and the categories varying

from study to study. Data on population variability in cycle length and length of the follicular and luteal phases are limited; a small number of studies on the patterns of hormone production, metabolism and excretion have been conducted (63). Therefore, although the menstrual cycle is commonly described as 28 days in length with variability greatest immediately after menarche and shortly before menopause and with considerably less variability exhibited from 20 to 40 years of age (63), a variety of 'normal' menstrual cycle length ranges have been suggested for healthy women (63, 168, 169, 170). This leads to difficulties in defining menstrual cycle dysfunction (171). Dysfunction at any level is capable of interfering with ovulation and preventing the fertilized egg from implanting (145). Abnormalities of menstruation include: oligomenorrhea, infrequent (>35 days) menstruation cycles; polymenorrhea, frequent (< 20 days) menstrual cycles; amenorrhea, absence of menstruation for > 90 days; menorrhagia, increased blood loss during menses; and metrorrhagia, irregular bleeding (79).

Pesticide Exposures and Menstrual Cycle Characteristics

Few epidemiologic studies have examined the relationship between exposure to pesticides and altered menstrual cycle characteristics. In a study of farm laborers, the most frequent medical complaint made by women attending migrant clinics was menstrual irregularity (28). The authors reported mean DDT levels were twice as high in women who complained of menstrual irregularities when compared with women who did not have this complaint, although the difference was not statistically significant (28). There was no difference in the frequency of menstrual-cycle irregularity between urban-born women and rural women (172). However, there was the potential for exposure

misclassification since area of residence was used as a proxy for pesticide exposure. The authors stated that direct measurements of exposure were needed to clarify exposure (172). In a prospective study of Laotian-born women of reproductive age, Windham et al. (27) reported a potential effect of dichlorodiphenyldichloroethylene (DDE) on ovarian function. A consistent association between various ovarian function outcome variables and DDT/DDE concentrations was observed (27). The authors reported an approximate 4 day shorter mean cycle length at the highest quartile concentration of DDT (95% CI: -7.8, 0.19) and the highest quartile of DDE (95% CI: -8.3, -.022) when compared with the lowest quartile (27). However, after adjustment for confounders and removal of a long cycle in the reference group (84 days), an imprecise association with a slightly reduced cycle length (< 1 day) was reported (27). In addition, women in the highest quartile of DDE (95% CI: -2.6, -0.20) and DDT (95% CI: -2.7, -0.30) had luteal phase lengths shorter by approximately 1.5 days (27). Furthermore, decreasing Pd3G during the luteal phase was also observed among these women (27). A major strength of this study was the use of biomarkers of both exposure and effect. Results of a 2005 cross-sectional study also suggested that DDT exposure was associated with a shortened menstrual cycle (26). However, a cross-sectional study of Chinese women by Chen et al. was not able to confirm these findings as neither DDE nor DDT was associated with menstrual cycle length, duration of menses or menstrual flow (152). These inconsistencies may be the result of the level of DDT being lower in the Chen et al. (152) study or their use of self-reported menstrual cycle length compared to Windham et al. (27) who used biomarkers to characterize menstrual cycles. Organochlorine exposure was found to be associated with permanent cessation of menstruation in a cross-sectional study of Hispanic women (173).

Women with DDT, hexachlorocyclohexane, or trans-nonachlor serum levels in the highest exposure categories experienced menopause 5.7, 3.4, and 5.2 years earlier, respectively, when compared with women with serum levels below the detection limit (173). Axmon et al. (21) also examined organochlorine exposure and menstrual cycle disruption and found that women exposed to organochlorines through fish consumption had 0.46 days (95% CI: 0.03 – 0.89) shorter menstrual cycles (21).

Atrazine and Menstrual Cycle Characteristics

To my knowledge, only one study has examined the relationship between atrazine and menstruation in humans. Farr et al. (25) found women who used either atrazine, lindane, or mancozeb reported long cycles (OR=2.7, 95% CI: 1.4, 5.2), missed periods (OR = 2.1, 95% CI: 1.4, 3.2), and intermenstrual bleeding (OR = 1.6, 95% CI: 1.1, 2.4) when compared with women who had never used these pesticides (25). Farr et al. (25) reported the pesticides with the strongest associations with menstrual cycle outcomes, lindane and atrazine, were the ones found to affect FSH and LH levels according to a detailed literature review of animal studies she conducted on 49 pesticides (79).

A limitation of this study was the potential for exposure misclassification due to temporality, since the assumption was made that lifetime pesticide was constant over time and is consistent with acute exposure (79). Recall bias may also have been present since menstrual cycle characteristics and exposures were assessed retrospectively via a questionnaire. The authors noted that use of menstrual cycle diaries would have reduced misclassification (79). In addition, information on pesticide use obtained via

questionnaires is usually not sufficient for valid dose-response assessment (51). Substantial exposure misclassification in dose-response analyses can result when depending on exposure metrics not validated with biomonitoring data (174). The prospective approach with the use of biomonitoring data is thought to be an effective way to improve the quality of pesticide exposure data (51, 174).

Other (Non-Pesticide) Exposures and Menstrual Cycle Characteristics

In a cross-sectional study of petrochemical workers in China, exposure to organic solvents (benzene, styrene, toluene, or xylene) was found to be associated with an increased frequency of oligomenorrhea (29). Each additional year of work was associated with a 7% increase in the risk of oligomenorrhea (OR = 1.07, 95% CI: 1.0, 1.14). An additional cross-sectional study among 3,000 petrochemical workers in China also reported a significant association between benzene exposure and abnormal menstrual cycle length, defined as an average cycle length of greater than 35 or less than 21 days, problems (30). The authors speculated that the biological pathway responsible for the abnormal menstrual cycle lengths is disruption of the hypothalamic-pituitary axis by altering GnRH frequency or amplitude (30). Windham et al. (33) found a consistent reduction in menstrual cycle length (-1.1 days, 95% CI: -1.8 to -0.40) and follicular phase length (-0.94 days, 95% CI: -1.6 to -0.24) among women exposed to chlorination by-products in drinking water. The authors suggested that a shorter follicular phase length reflects earlier ovulation, with the potential to disturb oocyte maturation, endometrial thickening, and conception (33). PCBs have been shown to significantly reduce cycle length by 1.11 days per month among women consuming more than one fish meal per

month (95% CI: –1.87, -0.35) (22). A strength of this study was the population-based design and use of nurses as interviewers, although a major limitation was the lack of information on potential confounders (22). Cooper et al. (23) also found a relationship between PCBs and menstrual cycle length, but this effect was to increase, not decrease, cycle length (p =0.02). Irregular cycles were also more frequent among those in the two highest PCB categories (OR = 1.5, 95% CI: 0.70, 3.3) (23). A population-based cohort study found dioxin exposure following an industrial explosion was associated with menstrual cycle length characteristics (24). Among women who were premenarcheal at the time of this 1976 industrial explosion in Seveso, Italy, a 10-fold increase in serum dioxin at the time was associated with menstrual cycles that were 0.93 days longer (95% CI: -.01, 1.86) when the women were studied 20 years later (24).

Menstrual Cycle Characteristic Assessment Methods

The two methods most commonly used to assess menstrual cycle characteristics in epidemiologic research are retrospective questionnaires (21, 22, 23, 24, 25, 26, 29, 30, 37, 40, 49, 151, 152, 158, 162, 163) and prospective diaries (2, 27, 33, 34, 36, 39, 40, 42, 44, 46, 48, 50, 153, 159, 161, 175, 176, 177, 178) with diaries sometimes used to complement biological monitoring (27, 33, 34, 46). To my knowledge, seven studies have compared retrospective and prospective menstrual cycle assessment, although each of these studies had limitations (153, 175, 178, 179, 180, 181, 182). Although prospective assessment can be limited by recruitment, compliance, drop-out rates, expense and the required time commitment, it has been suggested that prospective assessment is more accurate than retrospective assessment (175, 180) and, based on

Cohen's kappa statistic, agreement between the two methods is weak (181). Consequently, the validity of retrospective questionnaires to assess cycle length has been questioned.

Reliability of Retrospective Menstrual Cycle Data Assessed with Cohen's Kappa Statistic

Cohen's kappa is often the statistical measure used to quantify agreement beyond chance between two observers or two methodologies such as retrospective menstrual cycle questionnaires and prospective diaries. However, controversy exists over the use of Cohen's kappa as a single summary measure of agreement. Using Cohen's kappa without assessing the distribution of data across categories (i.e., normal versus not normal or "yes" and "no" responses) (183) or, in other words, a difference between cells *a* and *d* of the contingency table can under- or over-estimate reliability. In the case of menstrual cycle data, the more common a normal cycle is, the greater the likelihood of a low kappa statistic. Since the kappa statistic measures agreement beyond chance, and since chance agreement increases with an increase in the commonality of normal cycles, any agreement beyond chance becomes less probable. One can estimate the extent Cohen's kappa is affected by a large difference between normal and non-normal cycles by calculating the prevalence index (PI) (183).

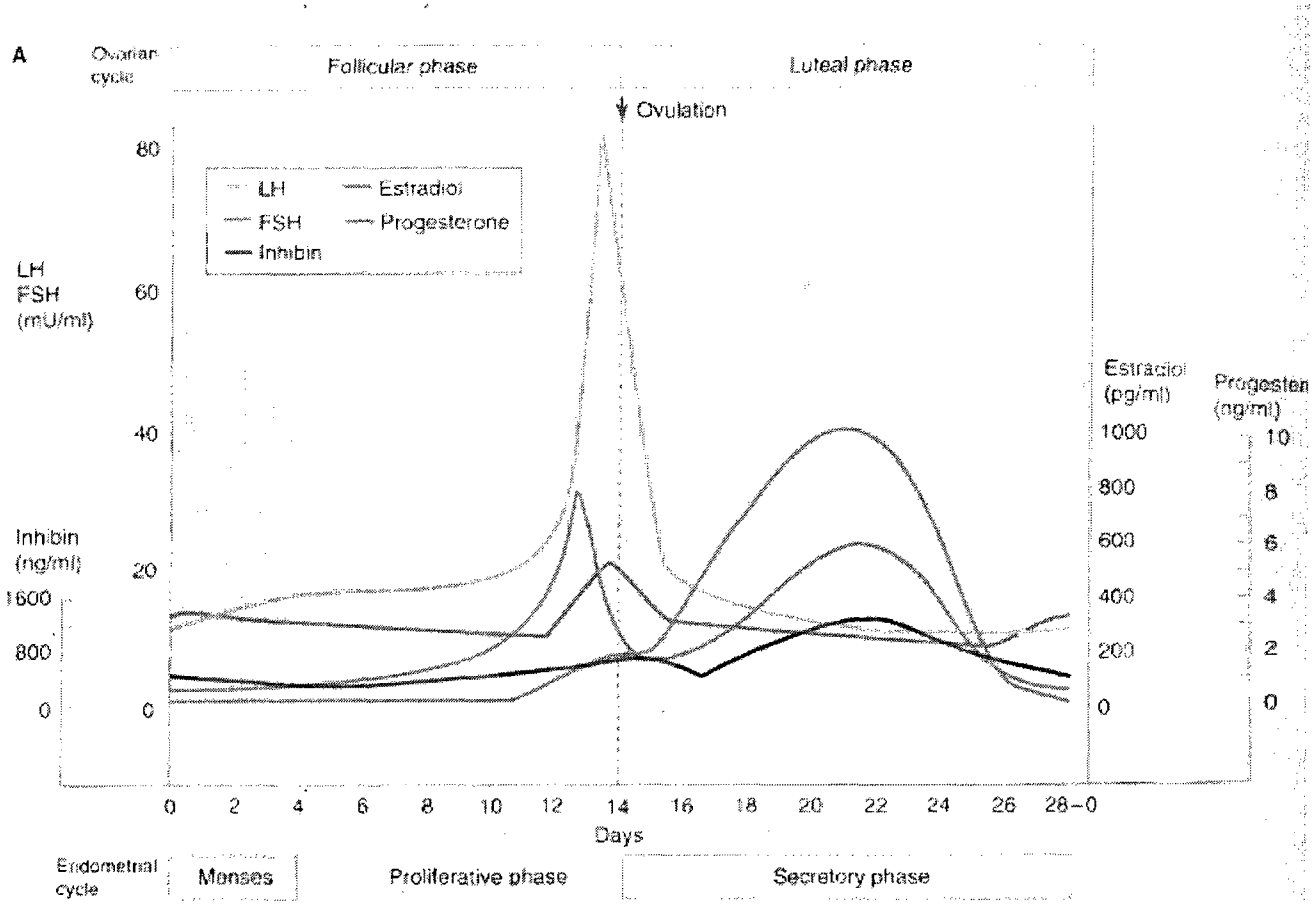


Figure 1.1. Hormonal changes during the menstrual cycle
(Adapted from Boron & Boulpaep 2003)

3. Research Design and Methods

Study Population

The state of Illinois was selected as the exposed study site location because of its intensive atrazine use (184). In Illinois in 2005, 87% of corn was treated with atrazine and more than 13,700,000 pounds of active ingredient applied (0.38 pounds active ingredient/acre in the state) (185). According to the EPA's National Pesticide in Drinking Water Wells Survey, atrazine exceeded the MCL of 3.0 ppb in eleven states, including Illinois (184). As required by the EPA's January 2003 Interim Reregistration Eligibility Decision (IRED), atrazine registrants (Syngenta Crop Protection, Inc., Dow Agrosciences, Drexel Chemical, Agan Chemical Manufacturing, Oxon Italia S.P.A. and Platte Chemical Company) must conduct drinking water monitoring in every surface water Community Water System (CWS) in the US where the annual average of the Safe Drinking Water Act data results in a value of 2.6 ppb or greater for atrazine and its chlorotriazine degradates (91). Because of this decision, 28 municipal water systems were monitored in Illinois by Syngenta Crop Protection, Inc. in 2005 (186). Mount Olive and Gillespie were two of the 28 systems and were selected for this study.

The state of Vermont was selected as the comparison site because of its low atrazine use. Unlike Illinois where corn grown in the state is used to supply the country, corn grown in Vermont is mainly used to feed Vermont dairy cows and, therefore, essentially stays in the state (C Giguere, personal communication, June 2008). In 2005, less than 51,000 pounds of active atrazine ingredient were applied throughout the entire state of Vermont

(0.009 pounds active ingredient/acre in the state), more than 250 times less than Illinois. (C Giguere, personal communication, June 2008).

Subject Selection and Exclusion Criteria

The study population was comprised of women 18 to 40 years old residing in Mount Olive and Gillespie, Illinois and a comparison group residing in Waterbury and Fair Haven, Vermont. Women were not eligible to participate if they had taken any form of hormonal contraception (oral, injectable or other medications for birth control) in the past 3 months; had used an intrauterine device (IUD) during the past 3 months; had used hormone replacements in the past 3 months; were pregnant or had been in the last 6 months; were breastfeeding or had breast-fed in the last 3 months; had surgery on reproductive tissues (except tubal ligation); or had been through menopause. Women were also not eligible to participate if they had been diagnosed with any of the following: chronic pelvic inflammatory disease, endometriosis, vaginal, cervical, uterine, or ovarian cancer, a pituitary tumor, acute hepatitis, human immunodeficiency virus infection or acquired immunodeficiency syndrome, hypothyroidism, hyperthyroidism, cirrhosis of the liver, hypopituitarism, Cushing's syndrome or diabetes. Exclusion criteria were developed to control for misclassification and potentially confounding since many of the above described conditions are associated with menstrual cycle disturbances and altered hormone levels.

Recruitment

The town clerks of the municipal offices of Mount Olive, Gillespie, Fair Haven and Waterbury provided contact information (name, address and phone number) for residences served by their respective water utility. Each residence first received a mailed letter explaining the study design, data collection procedures and informed consent. Subsequently, the investigator telephoned to determine whether an eligible woman lived at the residence and, if so, whether she was willing to participate.

There were three options for participation. To participate via option one, the women answered a questionnaire describing their menstrual cycle characteristics for the preceding year. The second option required women to answer a questionnaire and maintain a menstrual cycle diary. Women participating via option one or two were compensated $20. For the third option, women answered a questionnaire, maintained a menstrual cycle diary, collected daily urine samples through two menstrual periods until the third day after their second period ended and allowed a home tap water sample to be collected. They were compensated $50.

Outcome Selection

In a 1999 study of 598 menstrual cycles among women with no known fertility problems, Baird et al. (150) reported nonconception was associated with increased follicular phase length and the following hormonal patterns: reduced midluteal phase Pd3G, reduced preovulatory LH, reduced midluteal phase $E_1$3G and elevated follicular phase Pd3G. Therefore, these four reproductive hormones along with follicular phase length were

selected *a priori* as outcomes for this study. In addition, due to previously described toxicological findings of an inhibition of the LH surge and estrous cycle disruption and epidemiological findings of menstrual cycle disruption, LH surge levels and menstrual cycle characteristics (e.g., length regularity; length; spotting between menstrual periods; > six weeks without a menstrual period; and menstrual cramps) were also selected *a priori* as outcomes.

Menstrual cycle length irregularity was assessed by the question on the retrospective questionnaire, "Generally speaking, are your periods regular or irregular? That is, is the length of time between the first day of one period and the first day of the next about the same each cycle?" and categorical choices were yes or no. Severe menstrual cycle length irregularity (> six weeks/≤ six weeks without a menstrual period) was assessed by the following question: "During the last 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills." Menstrual cycle length was assessed using the following question on the retrospective questionnaire, "Many women have their periods about once a month. Some women have their periods more often and others less often. How often are your menstrual periods? In other words, how many days are there from the first day of one menstrual period to the first day of the next period?" The categorical response choices ≤ 24 days and 25-30 days were considered not long while 31-35, 36-42 and 43 days or more were considered long. Inter-menstrual bleeding (spotting between periods) was assessed by the question, "During the last 12 months did you ever bleed or spot between menstrual periods (Do not count times when you were pregnant, breast

feeding or using birth control pills/medication)?" and response choices were either yes or no. Experiencing menstrual cycle cramps was assessed by the question, "Approximately, how often do you have cramps or backache with your menstrual periods?" The categorical response choices 'never' and 'sometimes' were considered normal while 'often' and 'always' were considered not normal.

Data Collection

Questionnaire

Informed consent was obtained from each participant before beginning the initial interview following guidelines established by Colorado State University (CSU) Human Subjects Research Committee. Women participating via option one or two answered a questionnaire over the phone administered by a trained investigator at the time of the recruitment phone call. For women participating via option two, a menstrual cycle diary was mailed out immediately following completion of the questionnaire. For women providing urine and water samples and participating via option three, the questionnaire was administered by a trained investigator at the time of the home visit. The questionnaire took approximately 30 minutes to complete. Data were collected on potential confounders including: age, height, weight, race, education, income, occupation, proximity to a farm, use of chemicals at home, smoking, alcohol consumption, amount of caffeinated beverages consumed, fruit and vegetable consumption, supplement/vitamin consumption, hours of physical activity per week; exposure variables like use and type of filtration system for drinking and/or cooking, amount of water consumed daily both at home and at work, and the following reproductive health outcomes: menstrual cycle

characteristics, number of pregnancies, number of live births, and infertility. The questionnaire is available in Appendix A.

Menstrual Cycle Diaries

Diaries of daily menstrual activity were completed prospectively by women participating via option two or three (Figure 2.1). Women were asked to keep a diary through two menstrual bleeding periods until the third day after their second period ends. On a daily basis, women recorded the presence or absence and intensity or heaviness of bleeding using numbers denoting five levels (none, spotting, light, moderate, and heavy), presence or absence of menstrual backache or cramps and whether menstrual pain medication was taken. On a weekly basis, participants were asked to record whether they were sick, dieting or experienced weight loss, the number of hours of physical activity, the number of cigarettes or cigars smoked, the number of drinks of alcohol, and the number of cups of caffeinated beverages.

Collection of Urine Samples for Analyses of Atrazine and Atrazine Metabolites

Urine collection bottles provided by the CDC were prewashed following EPA methods using a laboratory grade, biodegradable, non-phosphate detergent, rinsed three times with tap water, rinsed again three times with deionized water, and oven-dried and packaged in organic-free conditions (187). A total of two first morning urine voids (one on each day) were collected two days apart for each participating woman. Urine was collected by the women in disposable screw cap collection cups and placed in a biohazard bag. Upon

receipt by the investigator, urine specimens were placed on ice in a cooler. Within 3 hours, 20 mL of urine were pipetted into a 30 mL clear glass bottle with a CDC bar-coded label. For creatinine (Cr) analysis, 1.8 mL of each participant's urine collection were pipetted into a 2 mL cryovial labeled with a CDC bar-coded label which corresponded with a CSU identification number. Aliquoted samples were frozen at -20° C immediately following sample preparation. All frozen urine samples were shipped on dry ice to the CDC laboratory (Dr. Dana Barr).

Collection of Residential Tap Water Samples for Analyses of Atrazine and Atrazine Degradation By-Products

Water collection bottles were also provided by the CDC and prewashed as previously described. For each participant, a total of four home tap water samples (two on each day) were collected two days apart for analysis of atrazine and atrazine degradation by-products. Cold tap water samples were collected in 15 mL amber glass bottles with Teflon screen caps marked with CDC bar-coded labels which corresponded with a CSU identification number. Before collection, the tap water was run for approximately two minutes allowing the system to flush and the water temperature to stabilize. The glass bottles were then filled about 2/3 full (10-12 mL). Following EPA methods to remove residual chlorine present in the municipal water, 4-5 mg of sodium sulfite was added to each duplicate water sample (187). This resulted in two water samples with chlorine and two without chlorine (sodium sulfite added). The water samples were placed on ice in a cooler for transport before being frozen at -20° C. All frozen water samples were shipped on dry ice to the CDC laboratory (Dr. Dana Barr).

Collection of Municipal Water Plant Monitoring Data for Determination of Atrazine and Atrazine Degradation By-Products

Atrazine and chlorotriazine levels at municipal plants in Illinois were monitored by Syngenta Crop Protection, Inc. (Wilmington, DE) on a weekly basis during the months of April, May, June and July and on a biweekly basis for the months of August, September and October in both Mount Olive and Gillespie, Illinois. The results of municipal plant analyses were obtained from the Illinois EPA Bureau of Water, Division of Public Water Supplies.

The municipal water supplies of Waterbury and Fair Haven, Vermont have waivers from the State of Vermont for monitoring synthetic organic chemicals (SOCs), including atrazine. Waivers are issued when SOCs have never been detected in a water supply and they continue to be administered as long as they do not have changes in land use in the source protection area. According to the Vermont Water Supply Division, it is expected that SOCs, including atrazine, will remain undetectable in municipal water in Fair Haven and Waterbury, Vermont. (J Siriano, personal communication, March 2006).

Collection of Urine Samples for Analyses of Reproductive Hormones and Determination of Follicular and Luteal Phase Lengths

Participants collected first morning urine voids daily for at least one entire menstrual cycle by collecting through two or more menstrual bleeding periods for determination of

levels of preovulatory LH, mid-luteal phase $E_1$3G, follicular phase Pd3G, mid-luteal phase Pd3G, LH peak, follicular phase length and luteal phase length.

Collection containers were provided by National Institute for Occupational Safety and Health (NIOSH). Urine samples were collected daily in sterile 250 mL cups and a portion poured, by the women, into 7 mL polypropylene vials. The vials contained glycerol to achieve a 7% dilution in urine to prevent loss of hormonal activity (188) and were pre-labeled with the participants NIOSH kit number and CSU identification number. The participants recorded the date and time of urine collection and stored their urine samples in provided boxes in their home freezer. At the end of collection, participants mailed the frozen urine samples surrounded by four ice packs in a Styrofoam chest to the NIOSH laboratory by next-day courier. Upon arrival, samples were stored at NIOSH at -80° until analysis.

Laboratory Analyses of Atrazine, Atrazine Metabolites, Atrazine Degradation By-Products, and Reproductive Hormones and the Determination of Follicular and Luteal Phase Lengths

Analyses of Urine and Water for Atrazine, Atrazine Metabolites, and Atrazine Degradation By-Products (CDC)

Atrazine degradation in water is slow with a half-life of more than 6 months (80). When biologically degraded in the environment, atrazine is typically dealkylated into desethylatrazine, desisopropyl atrazine, and diaminochlorotriazine (80, 93, 189). These

three metabolites can also be formed via metabolism in humans by the cytochrome P450 enzymes, specifically CYP1A2 (80, 190). Barr et al. (93) have diagramed the complex metabolism of atrazine (Figure 2.2). Atrazine is metabolized to three hydroxylated metabolites: hydroxyatrazine, hydroxy desethylatrazine and ammeline; and to four glutathione conjugated metabolites: atrazine mercapturate, DEAM, desisopropylatrazine mercapturate, diaminotriazine mercapturate. It was not expected that the glutathione-derived metabolites (the mercapturic acids) would be present in the drinking water samples since these metabolites must undergo phase II conjugation. The chlorinated atrazine degradates are considered to be equal in toxicity to their parent compound, atrazine.

Atrazine and its degradates and metabolites were analyzed in water and urine by the Organic Analytical Toxicology Branch Laboratory of the CDC (under the direction of Dr. Dana Barr). Previous analytic methods to detect atrazine and its metabolites have been limited by the number of metabolites detectable and the sensitivity of the analyses (191). Panuwet et al. (191) of the CDC have developed the most comprehensive and sensitive method with the ability to measure multiple atrazine biomarkers in urine and water.

In water, samples were collected and analyzed for atrazine, desethylatrazine, desisopropyl atrazine, and diaminochlorotriazine. In urine, samples were collected and analyzed for atrazine, desethylatrazine, desisopropyl atrazine, diaminochlorotriazine, atrazine mercapturate and DEAM. CDC was unable to measure the hydroxylated metabolites due to difficulty in keeping the standards stable enough for analyses (D. Barr,

personal communication, January 2008). Additionally, CDC was unable to measure two of the glutathione metabolites, desisopropylatrazine mercapturate and diaminotriazine mercapturate, because standards were not available for these metabolites.

Urine and water samples were analyzed in the same manner using an online solid phase extraction method coupled with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) by CDC (191). Atrazine degradates were analyzed using this analysis method without modification. The limit of detection was 0.5 ppb for atrazine, atrazine mercapturate, and DEAM and 1.0 ppb for desisopropylatrazine, desethylatrazine and diaminochlorotriazine. All analyses were conducted blind with respect to state of residence. Urinary atrazine and its metabolites were adjusted for Cr concentrations of the sample to normalize for sample dilution.

Analyses of Municipal Water Plant Monitoring for Determination of Atrazine and Atrazine Degradation By-Products (Syngenta Crop Protection, Inc.)

Municipal water samples were analyzed by Syngenta Crop Protection, Inc. using immunoassay (IA), gas chromatographic/mass selective detection (GC/MS) and liquid chromatographic/mass spectrometry/mass spectrometry detection (LC/MS) (186). According to Syngenta Crop Protection, Inc., until June 2005, water samples were run using IA with samples greater than 3.0 ppb run by GC/MS (B. Christensen, email to A. Rhodes, February 2009). After June 2005, all water samples were run using LC/MS (B. Christensen, email to A. Rhodes, February 2009). Results were reported as the atrazine concentration and the sum of atrazine and the three chlorotriazine degradate residues

(total chlorotriazine concentration). The limit of quantitation ranged from 0.05 to 0.50 ppb (186).

Analyses of Urinary Reproductive Hormones and Determination of Follicular and Luteal Phase Lengths (NIOSH)

Preovulatory LH level, mid-luteal phase $E_1$3G, follicular phase Pd3G, and mid-luteal phase Pd3G were analyzed in urine by the NIOSH Reproductive Endocrinology Laboratory. $E_1$3G is a metabolite of and correlates well with circulating estradiol, the most biologically active estrogen of the major estrogen metabolites (Figure 2.3). Pd3G is a metabolite of and correlates well with circulating progesterone. Reproductive hormone and cycle phase length measurements were based on an established algorithm centering around the day of luteal transition (DLT) (or luteinizing surge onset) (150). The DLT was defined as the first rise in LH > 2.5 times the mean level of the previous seven days. Preovulatory LH level was defined as the geometric mean of LH for the three consecutive days ending on the DLT. Mid-luteal phase $E_1$3G was defined as the geometric mean of $E_1$3G for days five and six after the DLT. Follicular phase Pd3G was defined as geometric mean of Pd3G from cycle day 5 through the third day before the DLT. Mid-luteal phase Pd3G was defined as geometric mean of Pd3G for days five and six after DLT and the LH peak was defined as the highest luteinizing value of the cycle that exceeded 8.5 mIU LH/mg Cr.

The follicular phase length was defined as the number of days from the first day of menses to the DLT. The luteal phase length was defined as the last day of the cycle

minus the day after the DLT.

LH levels were analyzed using commercial noncompetitive, two-site, time-resolved fluoroimmunoassays developed for analyses in urine as described by Kesner et al. (192). Standards and urine samples were measured in duplicate. Assay fluorescence was measured using a Perkin-Elmer Victor-2D multi-label counter. The major urinary metabolites of estradiol and progesterone, E13G and Pd3G, respectively, were assayed using competitive, double-antibody time-resolved fluoroimmunoassays developed and characterized by the NIOSH Reproductive Endocrinology Laboratory (193). Standards and urine samples were measured in triplicate and assay fluorescence was again measured using a Perkin-Elmer Victor-2D multi-label counter. All hormone concentrations were adjusted for Cr concentration.

Statistical Analyses

Specific Aim 1 - Participation

A flow chart outlining the number of participants for each level of participation by state was created.

Specific Aim 2 – Descriptive (study population)

Data were entered into a computerized database and all statistical analyses were performed using statistical analysis software (SAS) 9.1.3 (SAS Institute, Cary, NC). Descriptive statistics were calculated to summarize markers of exposure (state of residence, years in current home, consumption of unfiltered water), and potential

confounders and effect modifiers (ex. age, BMI, income, education, caffeine consumption, age at menarche, physical activity etc.). Mean and standard deviations (for continuous variables) and number and percent (for categorical variables) were calculated for the total population and stratified by state.

Specific Aim 3 – Descriptive (atrazine exposure)

Mean and standard deviations were calculated for the average of the two atrazine residential water samples (with chlorine and without chlorine), the average of the two chlorotriazine residential water samples (with chlorine and without chlorine) and the average of the two urinary DEAM samples. Mean and standard deviations were calculated for the total population and stratified by state. Since the assumptions of normality were not met, the non-parametric measure of correlation, the Spearman correlation, was conducted. Spearman correlation coefficients and p-values were calculated for the following: day 1 versus day 2 atrazine residential water samples with chlorine; day 1 versus day 2 atrazine residential water samples without chlorine; chlorine versus non-chlorine atrazine residential tap water for day 1; chlorine versus non-chlorine atrazine residential tap water for day 2; day 1 versus day 2 chlorotriazine residential water samples with chlorine; day 1 versus day 2 chlorotriazine residential water samples without chlorine; chlorine versus non-chlorine chlorotriazine residential tap water for day 1; and chlorine versus non-chlorine chlorotriazine residential tap water for day 2.

Specific Aim 4 – Descriptive (menstrual cycle characteristics)

Number and percents were calculated for the questionnaire data on menstrual cycle characteristics which included length regularity, long/not long, spotting between menstrual periods, going more than six weeks without a menstrual period and dysmenorrhea. Mean and standard deviation and number and percent were calculated for menstrual cycle diary data on menstrual cycle length. For menstrual cycle characteristics, number and percent (for categorical variables) and mean and standard deviation (for continuous variables) were calculated for the total population and stratified by state.

Specific Aim 5 – Descriptive (reproductive hormones and follicular phase length)

Means and standard deviations were calculated for the average of the two urinary samples analyzed for preovulatory LH, mid-luteal phase E13G, follicular phase Pd3G and mid-luteal phase Pd3G, LH peak, follicular phase length and luteal phase length. Means and standard deviations were calculated for the total population and stratified by state.

Specific Aim 6 – Atrazine exposure → questionnaire menstrual cycle characteristics

The goal of this analysis was to evaluate the relationship between markers of atrazine drinking water exposure and menstrual cycle characteristics as reported by retrospective questionnaire data. Crude and multivariable unconditional logistic regression were used to assess the relationship between atrazine exposure and the following dichotomous outcomes: regular/irregular menstrual cycle length; length of cycle (not long/long); spotting/not spotting between menstrual periods; > six weeks/≤ six weeks without a

menstrual period; and dysmenorrhea (never and sometimes/often and always) as previously descibed. Controls were women who reported regular menstrual cycle lengths, not long cycles, not spotting between menstrual periods, ≤ six weeks without a menstrual period and never or sometimes experiencing dysmenorrhea.

Associations were examined with the above described outcomes and the following markers of atrazine exposure: state of residence (Illinois women as exposed and Vermont women as unexposed); years in current home; and consumption of unfiltered water, assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?". The exposure variables years in current home and consumption of unfiltered water were dichotomized with cut-points determined by taking the median of the Illinois controls for each outcome individually. Since the variability between cut-points for each outcome was small, the most common cut-point for each exposure (years in current home and unfiltered water consumption) was then used for all five outcomes (regular/irregular menstrual cycle length; length of cycle (not long/long); spotting/not spotting between menstrual periods; > six weeks/≤ six weeks without a menstrual period; and dysmenorrhea (never and sometimes/often and always). Each exposure variable was assessed with and without Vermont women included as the unexposed population.

In an attempt to determine the background risk among women, an analysis was done for each of the markers of atrazine exposure among only Vermont women. Median cut-

points for years in current home and unfiltered water consumption used in the Illinois analyses were used for the Vermont analyses.

The following variables were evaluated for confounding: age (continuous), parity (nulliparous vs. parous), education (college graduate vs. non college graduate), income (<$60,000 vs. ≥ $60,000), caffeine (<300 mg/day vs. ≥300 mg/day), vegetable consumption (≤1 serving/day vs. >1 serving/day) and fruit consumption (≤1 serving/day vs. >1 serving/day), alcohol consumption (<1 drink/week vs. ≥ 1 drink/week), current smoking (yes/no), age at menarche (12-13 years/<12 vs. >13 years), amount of physical activity (≤ 2 hours/week vs. > 2 hours/week) and BMI (<25 vs. ≥25). Caffeine consumption was determined by multiplying the number of milligrams of caffeine (coffee = 107 mg, tea = 34 mg, cocoa = 10 mg and soda = 47 mg) by the number of cups, glasses or cans of each beverage consumed. BMI was calculated using the following formula: weight (pounds) / [height (inches)]^2 x 703. Weight categories associated with adult BMI ranges were obtained from the CDC (194). Cutpoints for confounders were based on previous findings in the literature or approximate median splits. As recommended by Kleinbaum et al. (195) confounding was evaluated based on a change in the effect estimate. With the exposure variable in the model, potentially confounding variables were first assessed individually and retained for the multivariable analysis if they changed the OR by 10% or more. All variables which changed the OR by 10% or more were then placed in the model together and removed one at a time to again evaluate for a 10% change in the OR. Confounders changing the OR by 10% or more remained in the final model. Confounding was not assessed if any of the exposure variables had less than

five women for any of the analyses since results would not have been reliable. Goodness of fit and interactions were not assessed due to small sample sizes. Frequencies, ORs, 95% CIs, and p-values were used in the construction of tables presented in the results section for this aim.

Specific Aim 7 – Atrazine exposure → diary cycle length

The goal of this analysis was to evaluate the relationship between drinking water exposure to atrazine (or markers of atrazine exposure) and menstrual cycle length (in days) as reported by the prospective menstrual cycle diary. Both crude and multivariable linear regression were used for the analyses.

The following markers of atrazine exposure (as described in Specific Aim 2) were examined: state of residence; years in current home; and consumption of unfiltered water. In order to estimate results below the limit of detection, all unvalued nondetectables were set to the LOD/$\sqrt{2}$ (196). The following atrazine exposure variables analyzed by CDC were examined: average of the two residential tap water atrazine concentrations with chlorine dichotomized at 0.36 (the atrazine limit of detection/$\sqrt{2}$); average of the two residential tap water atrazine concentrations with chlorine removed dichotomized at 0.36 (the atrazine limit of detection/$\sqrt{2}$); average of the two residential tap water chlorotriazine concentrations with chlorine dichotomized at 2.50 (the chlorotriazine limit of detection/$\sqrt{2}$); average of the two residential tap water chlorotriazine concentrations with chlorine removed dichotomized at 2.50 (the

chlorotriazine limit of detection/$\sqrt{2}$); and average of the two atrazine urinary biomarker DEAM samples dichotomized at 0.36 (the DEAM limit of detection/$\sqrt{2}$).

The results of the municipal plant monitoring (Syngenta Crop Protection, Inc.) for detection of atrazine in the municipal water systems of Mount Olive and Gillespie, Illinois were also used as exposure variables. Atrazine monitoring data were typically not available for each woman's exact date of participation, therefore, the two municipal plant (Syngenta Crop Protection, Inc.) results closest in time to the woman's date of participation were averaged and weighted according to their distance from the woman's participation date. This resulted in a temporally weighted average imputed for each woman. Final imputed values for both atrazine and chlorotriazine were dichotomized using a median split. A median split was used because no values were below the limit of detection.

In addition, the estimated 'dose' for both atrazine and chlorotriazine was used as an atrazine exposure variable. Estimated 'dose' was calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. The volume of unfiltered drinking water ingested was calculated from responses to the questionnaire question: "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?". Both the imputed atrazine and chlorotriazine municipal plant (Syngenta Crop Protection, Inc.) values and the atrazine and chlorotriazine residential tap water averages analyzed by CDC were used for the concentrations in the estimated 'dose' calculation.

The following variables were first assessed for confounding with the exposure of interest in the model and menstrual cycle length as the outcome: age, BMI, parity, current smoking status, weekly alcohol consumption, education, age at menarche, caffeine consumption, vegetable consumption, fruit consumption, income, and physical activity. With the exposure variable in the model, each potential confounder was entered into the model individually to determine its influence on the estimate of interest, the β coefficient for menstrual cycle length. Variables which changed the β coefficient in a meaningful manner were then placed in the model together and removed one at a time to re-evaluate for a change in the β coefficient. Any covariate which had a meaningful impact on the estimate of interest was retained in the final model.

Chi-square analysis was used to examine the associations between categorical variables indicating the potential for interdependence. Variables that were highly significantly associated ($p < 0.001$) with one another were not allowed in the same model.

The four assumptions, normality, independence, linearity and homoscedasticity, which justify the use of linear regression, were assessed. For the continuous dependent variable (menstrual cycle length), partial plots of standardized residuals versus menstrual cycle length were used to test linearity; plots of residuals versus predicted values were used to check homoscedasticity; and stem-and-leaf plots, boxplots, normal probability plots, and Shapiro-Wilk statistics were performed on the residuals in order to assess normality.

Interactions were not assessed due to small sample sizes. Frequencies, β coefficients (β), standard errors, 95% CIs, and p-values were used in the construction of tables presented in the results section for this aim.

Specific Aim 8 – Atrazine exposure → reproductive hormones and phase length

The goal of this analysis was to evaluate the potential relationship between drinking water exposure to atrazine (or markers of atrazine exposure) and the urinary concentrations of reproductive hormones. Crude and multivariable linear regression were used for the analyses.

The following markers of atrazine exposure, as described earlier, were examined: state of residence; years in current home; and consumption of unfiltered water. The following atrazine exposure variables, as described in Specific Aim 3, analyzed by CDC were examined: average of the two residential tap water atrazine concentrations with chlorine dichotomized at 0.36; average of the two residential tap water atrazine concentrations with chlorine removed dichotomized at 0.36; average of the two residential tap water chlorotriazine concentrations with chlorine dichotomized at 2.50; average of the two residential tap water chlorotriazine concentrations with chlorine removed dichotomized at 2.50; and the average of the two atrazine urinary biomarker DEAM samples dichotomized at 0.36. Imputed municipal plant monitoring (Syngenta Crop Protection, Inc.) results and 'dose' exposure variables were also used as atrazine exposure variables, as described earlier.

The following four reproductive hormones were assessed: preovulatory LH, mid-luteal phase E13G, follicular phase Pd3G and mid-luteal phase Pd3G. Previous research has found these reproductive hormones are associated with infertile ovulatory cycles (150). In addition, the following continuous variables were analyzed: follicular phase length, and level of the LH peak.

Again, the following variables were first assessed for confounding with the exposure of interest in the regression model and the particular reproductive hormone or phase length as the outcome: age, BMI, parity, current smoking status, weekly alcohol consumption, education, age at menarche, caffeine consumption, vegetable consumption, fruit consumption, income, and physical activity.

Baird et al. (150) reported conception was significantly associated with a one unit increase: preovulatory LH, mid-luteal phase $E_1$3G, follicular Pd3G, mid-luteal phase Pd3G and level of the LH peak (150). Therefore, a change in the estimate of interest, the β coefficient, from the linear regression model of greater than one unit was chosen as a clinically meaningful change for which to evaluate confounding. With the exposure variable in the model, variables were first assessed individually and retained for the multivariable analysis if the β coefficient changed by greater than one unit. Variables which changed the β coefficient by one unit or more were then placed in the model together and removed one at a time to re-evaluate for a change in the β coefficient. Confounders changing the β coefficient by more than one unit were then retained in the final model.

For phase length, each potential confounder was entered into the regression model individually with the exposure variable in the model to determine its influence on the estimate of interest, the β coefficient. Variables which changed the β coefficient in a meaningful manner were then placed in the model together and removed one at a time to re-evaluate for a change in the β coefficient. Any variable which changed the estimate of interest in a meaningful way was retained in the final model.

The assumptions which justified the use of linear regression were assessed as described in Specific Aim 3.

Interactions were not assessed due to small sample sizes. Frequencies, β coefficients, standard errors, 95% CIs, and p-values were used in the construction of tables presented in the results section for this aim.

Specific Aim 9 – Phase length and Pd3G → menstrual cycle characteristics

The goal of this analysis was to evaluate the already established association between phase length (luteal and follicular) and peak Pd3G with the following menstrual cycle characteristics: length, length regularity and > six weeks/ ≤ six weeks without a menstrual period. Crude and multivariable logistic regression were used for the analyses where length regularity and > six weeks/ ≤ six weeks without a menstrual period were the outcome variables. Crude and multivariable linear regression was used in the menstrual cycle length analysis.

The following independent variables, determined from diary data and urinary hormonal concentrations, were examined: follicular phase length, luteal phase length and peak Pd3G level.

The dichotomous dependent variables as reported by the retrospective questionnaire and analyzed using logistic regression were regular/irregular menstrual cycle length and > six weeks / ≤ six weeks without a menstrual period. Length regularity was assessed by the question, "Generally speaking, are your periods [cycles] regular or irregular? That is, is the length of time between the first day of one period and the first day of the other about the same each cycle?" Going more than six weeks without a menstrual period was assessed by the question, "During the last 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills." In addition, menstrual cycle length (in days) as reported by the prospective menstrual cycle diary was examined as a continuous variable using linear regression.

Confounding for dichotomous outcomes (logistic regression) was assessed as described in Specific Aim 2. For continuous outcomes (linear regression), confounding was assessed as described in Specific Aim 3.

Goodness of fit and interactions were not assessed due to small sample sizes. Frequencies, ORs, 95% CIs, and p-values for logistic analyses and frequencies, β

coefficients, standard errors, 95% CIs, and p-values for linear analyses were used in the construction of tables presented in the results section for this aim.

Specific Aim 10 – Comparison of retrospective and prospective menstrual cycle characteristics

The goal of this analysis was to compare retrospectively reported menstrual cycle characteristics with prospectively maintained menstrual cycle diary data. Percent agreement, Cohen's kappa and the PI were used for the statistical analyses. For the retrospective assessment of menstrual cycle characteristics, questionnaire responses on the normalcy of the menstrual cycle were used. To assess menstrual cycle normalcy, the following question was asked: "Many women have their periods about once a month. Some women have their periods more often and others less often. How often are your menstrual periods? In other words, how many days are there from the first day of one menstrual period to the first day of the next period?" The categorical response choices were 24 days or less, 25-30 days, 31-35 days, 36-42 days, 43 days or more, too irregular to say and don't know.

For the prospective assessment of menstrual cycle characteristics, women maintained a diary through two menstrual bleeding periods and continued for three days after their second period ended. Bleeding or spotting activity was recorded on a daily basis. Since it is uncertain how a normal menstrual cycle length should be defined, we used two different definitions, every 25-30 days (definition 1) and every 25-35 days (definition 2).

Overall percent agreement for both definition 1 and 2 was calculated by dividing the number of concordant pairs (women in agreement on both their retrospective questionnaire and their prospective diary) by the number of concordant and discordant pairs. Chance-adjusted agreement between retrospective questionnaires and prospective diaries was evaluated using Cohen's kappa. Benchmarks provided by Landis and Koch(197) were used to evaluate the strength of agreement. The PI was calculated to evaluate the influence of differences between the prevalences of normal and non-normal cycle length on Cohen's kappa. The PI ranges from -1 (no non-normal cycles) to 1(no normal cycles) and is equal to 0 when the distribution across categories (i.e., normal versus not normal) is equally probable. The further the PI is from 0, the greater the influence of prevalence on Cohen's kappa. The PI is estimated by $\frac{(a-d)}{N}$ (183). A higher PI results in a lower kappa. Demographic characteristics were compared between women whose prospective and retrospective data were concordant and women whose prospective and retrospective data were discordant. ORs and 95% CIs were used for analysis of demographic characteristics. Means and standard deviations were calculated.

Statistical Power

Power calculations were performed during the study design phase and again after data collection. Power calculations used in the construction of Tables 2.1 and 2.2 were calculated based on the sample size determined after data collection.

Power calculations were computed for a dichotomous outcome, abnormal versus normal menstrual cycle and a binary exposure. A 30% prevalence of menstrual cycle abnormalities as reported in a cross sectional study among randomly selected Danish citizens by Munster et al. (198) was used.

Based on a sample size of 102 women, an alpha of 0.05, 80% power, a 1:1 ratio of unexposed to exposed, and a 30% prevalence of disease among the unexposed, an OR of 3.4 could be detected (Epi Info, version 3.3.2). This study had 61% power to detect an OR of 2.7, the OR reported by Farr et al. (25) among agricultural health study participants using hormonally active pesticides (including atrazine) and experiencing long menstrual cycles (Table 2.1). A limitation of this power determination method was that it used a univariate assessment and, was, therefore, not able to account for potential confounders. Power is expected to decrease with multivariable assessment and with changes to the ratio of unexposed to exposed.

Because of the limited literature available, power could not be calculated according to the reported difference in LH levels in women with pesticide (or specifically atrazine) exposure in drinking water when compared to those without pesticide (or atrazine) exposure in drinking water. Instead, differences were based on a study assessing the effects of fuel and solvent exposure on menstrual function. Reutman et al. (199) reported a difference of 7.2 mIU/mg Cr in mean preovulatory LH levels in women in the US Air Force with high aliphatic hydrocarbon breath levels as compared to women with low aliphatic levels.

When LH was examined as a continuous variable and atrazine as a dichotomous variable, a two-group independent sample t-test was used to estimate the power. Calculations were based on a sample size of 36 women and an alpha of 0.05 (Table 2.2). According to Table 2.2, this study had 55% power to detect a difference of 7.2 mIU/mg Cr in mean preovulatory LH, the difference detected by Reutman et al. (199). A difference of 9.2 mIU/mg Cr mean LH could have been detected with 76% power. This power determination method was also based on univariate associations.

Table 2.1. Power to detect menstrual cycle abnormalities ($\alpha = .05$; n=102)

	Odds Ratio		
	2.0	2.7	3.0
% Power	31%	61%	70%

Table 2.2: Power to detect change in preovulatory luteinizing hormone ($\alpha = .05$; n=36)

	Change in mean Preovulatory Luteinizing Hormone		
	7.2 mIU/mg Cr	9.2 mIU/mg Cr	11.2 mIU/mg Cr
% Power	55%	76%	90%

Cr, creatinine

	Mon 11	Tue 12	Wed 13	Thu 14	Fri 15	Sat 16	Sun 17
Answer every day:							
Bleeding or spotting 0=no, 1=yes	☐	☐	☐	☐	☐	☐	☐
Amount of blood 0=none, 1=spotting, 2=light, 3=moderate, 4=heavy	☐	☐	☐	☐	☐	☐	☐
Menstrual backache or cramps 0=no, 1=yes	☐	☐	☐	☐	☐	☐	☐
Medication for menstrual pain 0=no, 1=yes	☐	☐	☐	☐	☐	☐	☐
Today's urine collection vial number	☐	☐	☐	☐	☐	☐	☐
Time of today's urine sample collection	☐	☐	☐	☐	☐	☐	☐

Answer at the end of the week:

Were you sick this week? ☐
0=no, 1=yes

How many hours this week did you do heavy work or exercise that increased your breathing or heart rate? ☐
fill in total number

Were you dieting to lose weight? ☐
0=no, 1=yes

How many cigarettes or cigars did you smoke this week? ☐
fill in total number

How many drinks of alcohol (beer, wine, liquor) did you have this week? ☐
fill in total number

How many cups of caffeinated coffee, tea and soft drinks did you have this week? ☐
fill in total number

Please turn over to record medications, any problems with urine collection and if you were sick.

Figure 2.1. Menstrual cycle diary example

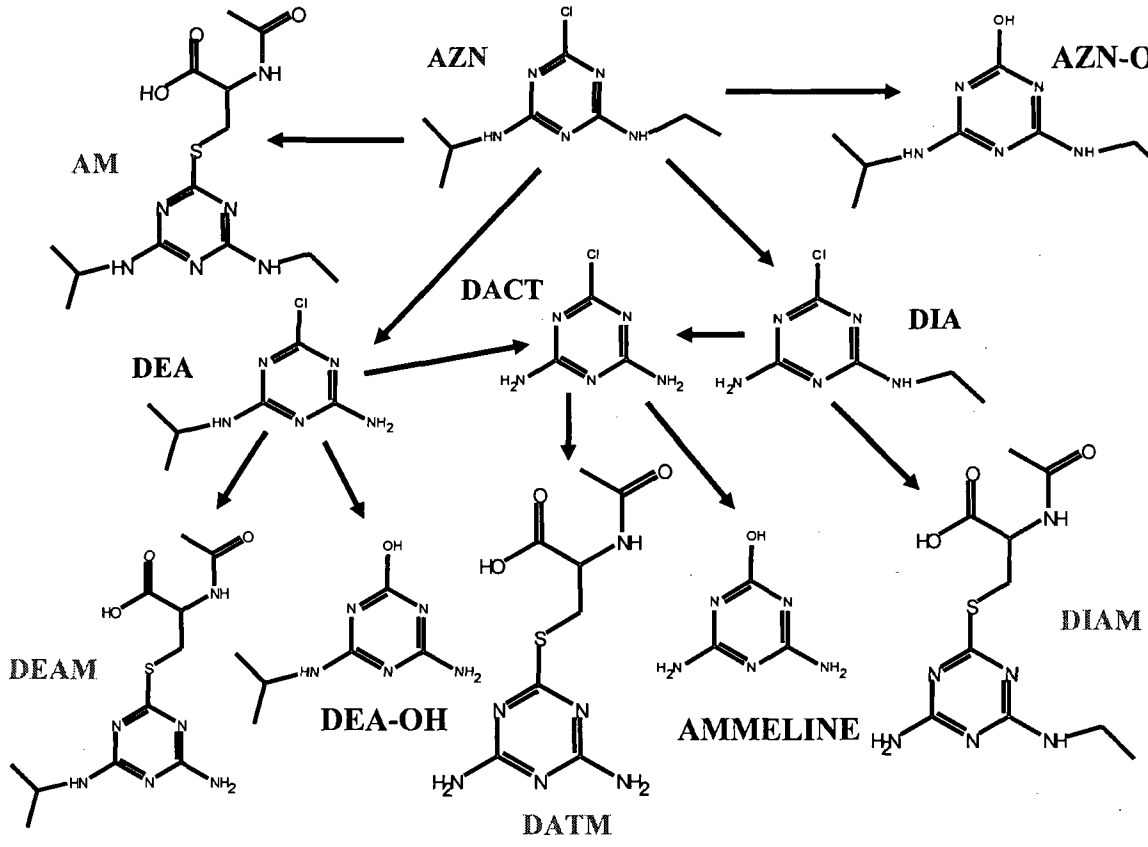


Figure 2.2. Metabolism of atrazine. Atrazine is labeled in red; dealkylated metabolites are labeled in black; hydroxylated metabolites are labeled in blue; and glutathione-derived mercapturic acid metabolites are labeled in green. AZN, atrazine; AZN-OH, hydroxyatrazine; AM, atrazine mercapturate; DACT, diaminochlorotriazine; DEA desethylatrazine; DEAM, desethylatrazine mercapturate; DEA-OH, hydroxydesethylatrazine; DIA, desisopropyl atrazine; DIAM, desisopropylatrazine mercapturate. (Adapted from Barr et al. 2007)

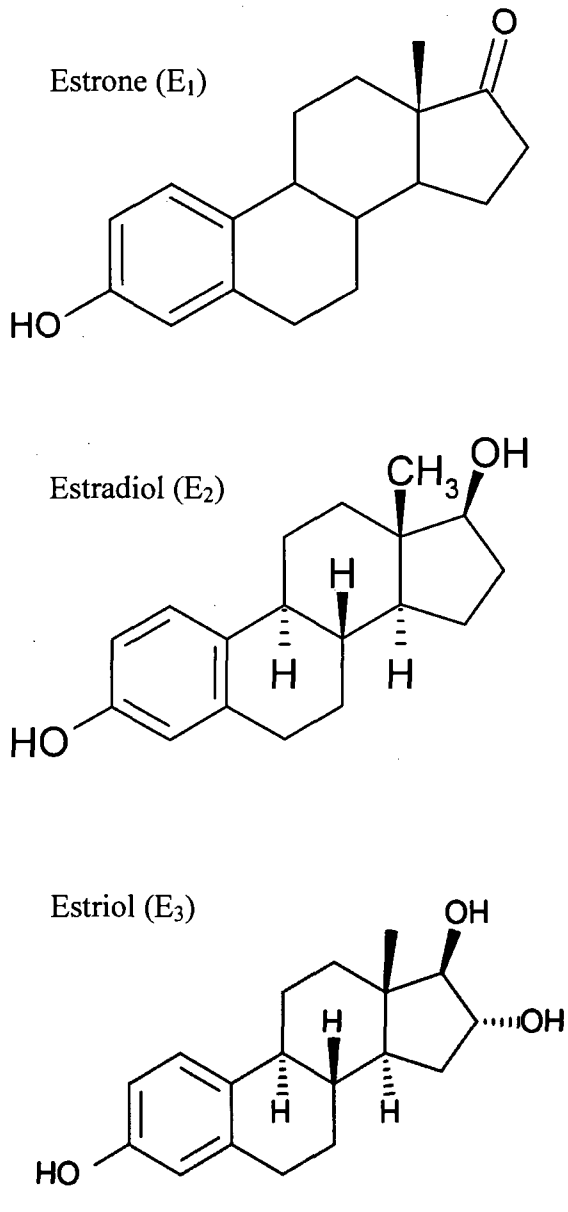


Figure 2.3. Major estrogen metabolites
(Boron & Boulpaep)

4. Results

Specific Aim 1 – Participation

According to the 2000 census, the population size of Mount Olive and Gillespie, Illinois wass 2,150 and 3,412, respectively. The population size of Waterbury and Fair Haven, Vermont was 4,915 and 2,928, respectively. A total of 1,826 recruitment phone calls were made (976 in Illinois and 850 in Vermont). Of the recruitment call attempts, 184 (162 in Illinois and 22 in Vermont) resulted in nonworking phone numbers and 402 (159 in Illinois and 243 in Vermont) failed to reach an individual. The majority of individuals contacted were not eligible. No eligible participants resided at 1,022 (82.42%) (519 in Illinois and 503 in Vermont) of the remaining 1,240 households called. In Illinois, 519 (79.24%) of 655 contacted individuals were not eligible. In Vermont, 503 (86%) of 585 contacted individuals were not eligible. Specific reasons of ineligibility were not captured. Refusal rates were statistically different between Vermont and Illinois (p = 0.003). In Illinois, 83 (61.03%) of the remaining 136 eligible women refused to participate. In Vermont, 33 (40.24%) of the remaining 82 eligible women refused to participate. Demographic data were not obtained for nonparticipating women, therefore, it is not known how Vermont and Illinois nonparticipating women differed. The overall participation rates (i.e., number of individuals who participated out of the number of phone calls made) did not differ by state (p = 0.76) (Illinois = 5.43% compared to 5.76% in Vermont).

One hundred and two women agreed to participate in the study by answering a retrospective questionnaire (53 Illinois women and 49 Vermont women) (Figure 3.1).

Sixty seven women (65.69%) maintained menstrual cycle diaries and answered questionnaires (30 Illinois women and 37 Vermont women). Thirty nine women (38.24%) provided daily urine samples for hormone analyses, maintained diaries and answered questionnaires (18 Illinois women and 21 Vermont women).

Specific Aim 2 – Descriptive (study population)

Personal characteristics are presented in Table 3.1 for the total study population and stratified by state. Vermont women were older than Illinois women (35.06 years vs. 32.62 years, $p = 0.01$) and more educated (48.98% college graduates compared to 30.19%, $p = 0.05$). There was no statistically significant difference between Vermont and Illinois women with regards to consumption of one or more alcoholic drinks per week (53.06% compared to 37.74%); more than one serving of fruit (38.78% compared to 37.74%); more than one serving of vegetables (65.31% compared to 60.38%), and 300 or more mg/day of caffeine (24.53% compared to 16.33%). There was also no statistically significant difference between Vermont and Illinois women with regards to high BMI (25.37% compared to 27.21%); proportion of current smokers (18.37% compared to 30.19%); exercising more than 3 hours per week (47.17% compared to 53.06%); and having a household income of $60,000 or more (29.79% compared to 35.42%). None of the women reported currently working with pesticides or herbicides at her job, although one woman described working with them in the past as a veterinary technician. Two women from Illinois reported working on a farm or in agriculture; however, neither collected urine samples for hormone analysis.

Reproductive characteristics are presented in Table 3.2 for the total study population and stratified by state. The mean number of pregnancies was similar for Vermont women (2.12 pregnancies) compared to Illinois women (2.42 pregnancies) (p = 0.37). The number of live births was also similar between Vermont women (1.59 births) and Illinois women (1.79 births) (p = 0.39). While not statistically significant, a higher proportion of Vermont women conceived in less than one year (76.74% compared to 68.85%, p = 0.27).

Specific Aim 3 – Descriptive (atrazine exposure)

Residential Monitoring - water

Residential water samples were obtained from tap water in 39 homes (18 homes in Illinois and 21 homes in Vermont). Overall, no concentrations of atrazine in water were at or above the EPA MCL of 3.0 ppb. This was true of the residential tap water samples regardless if chlorine was present or not (Tables 3.3 and 3.4). Less than 45% had atrazine concentrations above the limit of detection. The mean atrazine tap water concentration with chlorine was 0.61 ppb and without chlorine it was 0.53 ppb, 5 times lower than the EPA MCL. No tap water sample contained more than 1.91 ppb of atrazine.

To account for few results being above the limit of detection in water, atrazine and the chlorinated metabolites of atrazine (desisopropylatrazine, desethylatrazine and diaminochlorotriazine) were combined to make a chlorotriazine exposure variable. Ultimately, the two categories for residential drinking water were atrazine and

chlorotriazine. The two days atrazine residential water samples were averaged and the two days chlorotriazine water samples were averaged. This was done separately for samples with and without chlorine.

Spearman correlation coefficients and p-values for day 1 versus day 2 atrazine residential water samples with chlorine; day 1 versus day 2 atrazine residential water samples without chlorine; chlorine versus non-chlorine atrazine residential tap water for day 1; chlorine versus non-chlorine atrazine residential tap water for day 2; day 1 versus day 2 chlorotriazine residential water samples with chlorine; day 1 versus day 2 chlorotriazine residential water samples without chlorine; chlorine versus non-chlorine chlorotriazine residential tap water for day 1; and chlorine versus non-chlorine chlorotriazine residential tap water for day 2 are presented in Table 3.5. Day 1 atrazine residential tap water concentrations with chlorine were highly correlated with day 2 atrazine residential tap water concentrations with chlorine (Spearman correlation coefficient = 0.81; p = <0.0001). Day 1 atrazine residential tap water concentrations without chlorine were highly correlated with day 2 atrazine residential tap water concentrations without chlorine (Spearman correlation coefficient = 0.78; p = <0.0001). Day 1 atrazine residential tap water samples with chlorine were highly correlated with day 1 atrazine residential tap water samples without chlorine (Spearman correlation coefficient = 0.87; p = <0.0001). Day 2 atrazine residential tap water samples with chlorine were highly correlated with day 2 atrazine residential tap water samples without chlorine (Spearman correlation coefficient = 0.95; p = <0.0001). Chlorotriazine concentrations were low to moderately correlated (Table 3.5).

The mean and standard deviation of the two averaged atrazine and chlorotriazine concentrations in water (with chlorine and without chlorine) are presented in Table 3.6 for the total population and stratified by state. Atrazine concentrations (with and without chlorine) were higher among Illinois homes as compared to Vermont homes ($p = < 0.001$, both with chlorine and without chlorine) but only three times higher. In spite of an elevation in Illinois, tap water concentrations remained three times lower than the EPA MCL. Chlorotriazine tap water concentrations were not different between Illinois and Vermont homes ($p = 0.22$ with chlorine and $p = 0.28$ without chlorine). In fact, the mean chlorotriazine tap water concentration without chlorine was higher in Vermont (3.25 ppb) as compared to Illinois (2.48 ppb). In sum, tap water atrazine and chlorotriazine concentrations in Vermont and Illinois were below the EPA MCL, atrazine concentrations were higher in Illinois and chlorotriazine concentrations were similar in both states.

Municipal Plant Monitoring – water

As required by the EPA, Syngenta Crop Protection, Inc. developed an atrazine monitoring program to monitor community water at the municipal plants in Mount Olive and Gillespie, Illinois. According to their 2003, 2004, 2005 and 2007 monitoring results, community drinking water annual average levels for both atrazine and chlorotriazine were lowest in 2005 (data for 2006 were not available from the Illinois EPA at the time this was written)(Figures 3.2 and 3.3). In 2005, according to municipal plant monitoring (Syngenta Crop Protection, Inc.), atrazine averaged 0.21 ppb in Mount Olive and 0.29

ppb in Gillespie while chlorotriazine levels averaged 0.51 ppb in Mount Olive and 0.73 ppb in Gillespie (Tables 3.7 - 3.10). Atrazine 2005 levels ranged from 0.05 ppb to 1.17 ppb in both Gillespie and Mount Olive. Chlorotriazine 2005 levels ranged from 0.39 ppb to 2.08 ppb in Gillespie and from 0.20 to 1.86 in Mount Olive. The 2005 atrazine average during the study time period (July 13, 2005 to September 18, 2005) was 0.16 ppb in Mount Olive and 0.36 ppb in Gillespie. The 2005 chlorotriazine levels during the study period were 0.53 ppb in Mount Olive and 0.96 ppb in Gillespie.

Municipal plant annual average 2003 levels were 20 times as high in Mount Olive as compared to the 2005 Mount Olive levels. Annual average 2003 levels were twice as high in Gillespie as compared to the 2005 levels. In 2003, average annual atrazine levels were 4.28 ppb in Mount Olive and 0.64 ppb in Gillespie while chlorotriazine average levels were 5.30 ppb in Mount Olive and 1.08 ppb in Gillespie. The 2003 atrazine levels ranged from 0.71 ppb to 9.83 ppb in Mount Olive. In Gillespie, atrazine levels ranged from 0.07 ppb to 1.18 ppb. Chlorotriazine 2003 levels ranged from 1.04 ppb to 11.86 ppb in Mount Olive and from 0.20 ppb to 1.75 ppb in Gillespie.

Chlorotriazine and atrazine levels in 2004 were also twice as high as 2005 levels. Municipal plant atrazine levels averaged 1.57 ppb in Mount Olive and 0.65 ppb in Gillespie in 2004. Chlorotriazine annual average levels were 2.57 ppb in Mount Olive and 1.23 ppb in Gillespie in 2004. The 2004 atrazine community drinking water levels ranged from 0.79 ppb to 4.00 ppb in Mount Olive and from 0.03 ppb to 1.58 ppb in

Gillespie. Chlorotriazine levels ranged from 1.11 ppb to 5.20 ppb in Mount Olive and from 0.14 ppb to 2.70 ppb in Gillespie.

Correlations between imputed municipal plant atrazine concentrations and residential tap water atrazine concentrations were weak and not statistically significant (Spearman correlation coefficient = 0.04; $p = 0.89$) (Table 3.11). Correlations for imputed municipal plant chlorotriazine concentrations and residential tap water chlorotriazine concentrations were also low and not statistically significant (Spearman correlation coefficient = 0.18; $p = 0.51$) (Table 3.11).

Municipal Monitoring – water – Illinois EPA

Municipal plants are required to monitor atrazine drinking water concentrations at the plant and report results on a quarterly basis to the Illinois EPA. According to the Illinois EPA community drinking water data, atrazine concentrations were much lower in 2005 than the immediately preceding or immediately following years (Figures 3.4 and 3.5). In 2003, the average atrazine municipal plant concentration was 0.50 ppb in Gillespie and 2.08 ppb in Mount Olive. In 2004, the average atrazine municipal plant concentration was 0.23 ppb in Gillespie and 1.13 ppb in Mount Olive. In 2005, no water sample in either Mount Olive or Gillespie exceeded the limit of detection. This resulted in average atrazine concentrations of 0.0 ppb in both Gillespie and Mount Olive for 2005. Average atrazine concentrations remained at 0.0 ppb in Mount Olive in 2006 but increased to 0.37 ppb in Gillespie. In 2007, atrazine averages were 1.48 ppb in Mount Olive and 0.40 in Gillespie.

Urinary Monitoring

Data for urinary concentrations of atrazine and atrazine metabolites are presented in Table 3.12. The majority of atrazine concentrations in urine were at or below the limit of detection. Only DEAM had more than 16% of urine samples above the limit of detection. Therefore, only DEAM was examined as a urinary exposure variable. DEAM levels from the two consecutive days collections were averaged. Correlations between day 1 versus day 2 DEAM concentrations were weak and not statistically significant (Spearman correlation coefficient = 0.23; $p = 0.16$) (Table 3.5). Urinary DEAM levels (mean and standard deviation) for the total population and stratified by state are presented in Table 3.13. The average DEAM concentration was almost two times higher in Vermont (14.44 ppb) compared to Illinois (7.89 ppb) although the difference was not statistically significant ($p = 0.57$). It should also be noted that the standard deviations were high in the Vermont and the Illinois populations which was most likely due to a couple of outliers.

Years in Home, Water Consumption and Atrazine Estimated 'Dose'

Data for exposure surrogates are presented in Table 3.14. There was no statistically significant difference between Vermont and Illinois women for the number of years they lived in their current homes as reported on the retrospective questionnaire (6.12 years for Vermont women compared to 6.66 years for Illinois women) ($p = 0.57$). There was also no statistically significant difference between Vermont and Illinois women and their total average water consumption (8.20 cups per day year for Vermont women compared to

7.54 cups/day for Illinois women) (p = 0.44). Accounting for only unfiltered water consumption, there was no statistically significant difference between Vermont and Illinois women (2.02 cups/day year for Vermont women compared to 1.98 cups/day for Illinois women) (p = 0.93). Using unfiltered water consumption and the atrazine concentration in tap water to calculate estimated 'dose', there was no statistically significant difference between Illinois and Vermont women (estimated 'dose' (CDC) was greater than 0.36 ppb in 61.9% of Vermont women compared to 82.4% of Illinois women) (p = 0.17). There was also no statistically significant difference between Illinois and Vermont women and for estimated chlorotriazine 'dose' (estimated chlorotriazine 'dose' (CDC) was greater than 2.50 ppb in 76.2% of Vermont women compared to 82.4% of Illinois women) (p = 0.70).

Specific Aim 4 – Descriptive (menstrual cycle characteristics)

Menstrual cycle characteristics are presented in Table 3.15 for the total study population and stratified by state. According to prospective menstrual diaries, Vermont and Illinois women had similar menstrual cycle lengths (30.5 days for Vermont women vs. 32.3 days for Illinois women) (p = 0.50). According to retrospective questionnaire data, the following were similar between Vermont and Illinois women: inter-menstrual bleeding (10.2% of Vermont women compared to 15.1% of Illinois women) (p = 0.46); cramping (67.4% of Vermont women compared to 56.6% of Illinois women) (p = 0.26); age at menarche (60.4% of Vermont women were between the ages of 12 and 13 compared to 57.1% of Illinois women) (p = 0.74); and long cycle length (31 days or more) (14.9% of Vermont women compared to 20.0% of Illinois women) (p = 0.51). Menstrual cycle

length regularity was statistically different between Vermont and Illinois women with Vermont women more likely to report regular length menstrual cycles (89.6% of Vermont women compared to 64.7% of Illinois women) (p = 0.003). Consistent with length regularity, Vermont women were more likely to predict the onset of their period within four days (81.3% of Vermont women compared to 64.2% of Illinois women) (p = 0.06). Illinois women were more likely to go more than six weeks without a menstrual period (20.8% of Illinois women compared to 4.1% of Vermont women) (p = 0.01).

Specific Aim 5 – Descriptive (reproductive hormones and phase lengths)

Urinary hormone levels and menstrual phase lengths (mean and standard deviation) are presented in Table 3.16. In general, there is a great deal of variation in the reproductive hormones and menstrual cycle characteristics of women considered 'normal', and, therefore, it can be difficult to discern variation that exceeds normal ranges or that represent biologically meaningful changes. At the risk of over-interpreting the hormone results, it can be stated that reproductive hormone levels were in general agreement with those in the literature (150). Baird et al. (150) reported the following mean hormone values for cycles where conception occurred with the corresponding mean values for this study in parentheses: Preovulatory LH level – 15.2 mIU/mg Cr (17.2 mIU/mg Cr); mid-luteal phase $E_1$3G – 39.6 ng/mg Cr (28.26 ng/mg Cr); follicular phase Pd3G – 0.53 µg/mg Cr (0.91 µg/mg Cr); and mid-luteal phase Pd3G – 4.4 (10.42 µg/mg Cr).

There were no statistically significant differences by state for any of the hormones or follicular phase length. Levels for all hormones were consistently higher in Vermont

women, although not statistically higher. Mean preovulatory LH levels were higher in Vermont (18.45 mIU/mg Cr) as compared to Illinois (15.77 mIU/mg Cr) (p = 0.50). Levels of LH surge were also higher in Vermont (55.02 mIU/mg Cr) compared to Illinois (49.27 mIU/mg Cr) (p = 0.42). Of all the hormones, the difference in mid-luteal phase $E_1$3G levels between Vermont and Illinois was closest to significance (31.34 ng/mg Cr compared to 24.15 ng/mg Cr; p = 0.11). Levels were higher in Vermont as compared to Illinois for both follicular phase Pd3G (Vermont = 0.98 μg/mg Cr; Illinois = 0.82 μg/mg Cr) and mid-luteal phase Pd3G hormone levels (Vermont = 10.93 μg/mg Cr; Illinois = 9.73 μg/mg Cr), but not statistically higher (p = 0.51 for both follicular phase Pd3G and mid-luteal phase Pd3G).

Follicular phase length was longer in Vermont women (16.0 days) as compared to Illinois women (15.87 days) but not significantly longer (p = 0.94). Luteal phase length was slightly longer in Vermont women (12.95 days) as compared to Illinois women (12.87 days), and not statistically significant (p = 0.88).

Specific Aim 6 – Atrazine exposure → questionnaire menstrual cycle characteristics

The intention of this analysis was to assess the relationship between menstrual cycle characteristics, as reported by the retrospective questionnaire, and markers of atrazine drinking water exposure.

The menstrual cycle characteristics length regularity, length, spotting, cramps, and going more than six weeks without a menstrual period were assessed retrospectively from

questionnaires provided by 102 women. The cut-point selected for years in current home was 4 years. The cut-point for unfiltered water consumption was 2 glasses/day. For each menstrual cycle characteristic outcome, an attempt was made to determine the background risk by conducting analyses among Vermont women only. Results of analyses for Illinois and Vermont women were examined to determine if there was a difference in magnitude or statistical significance from the background risk. Crude and adjusted ORs, 95% CIs, and p-values are presented in Tables 3.17 through 3.21 and described below.

Menstrual Cycle Length Irregularity

Menstrual cycle length irregularity was assessed by the question, "Generally speaking, are your periods regular or irregular? That is, is the length of time between the first day of one period and the first day of the next about the same each cycle?". Crude and adjusted ORs, 95% CIs, and p-values for menstrual cycle length irregularity and the various markers of atrazine exposure are presented in Table 3.17. A statistically significant association (OR = 4.69; 95% CI: 1.58 - 13.95) was observed between state of residence and menstrual cycle length irregularity, Illinois as exposed and Vermont as unexposed. This association remained after adjusting for age and BMI (OR = 4.45 95% CI: 1.32 – 15.01).

No statistically significant associations were observed between years in current home as the exposure and menstrual cycle length irregularity among only Vermont women (background risk). Results, however, were statistically significant and ORs increased in

magnitude when Illinois women were included as exposed. A significant association (OR 6.88; 95% CI: 2.08 – 22.78) was observed between cycle length regularity and residing more than 4 years in current home when Illinois women were considered exposed and Vermont women unexposed. As years in current home increased among Illinois women, increasing ORs were observed in a dose response manner although CIs were wide. Statistically significant associations remained in the multivariable models adjusting for age, BMI and income (OR = 8.55, 95% CI: 2.16 – 33.96).

No statistically significant association was observed between unfiltered water consumption and menstrual cycle length irregularity for Vermont women only (background risk) (OR = 1.38, 95% CI: 0.21 – 9.23). Among Vermont and Illinois women, ORs increased in possible dose response manner as unfiltered water consumption increased, although statistically significant, the CIs were wide ($\leq$ 2 cups OR = 4.10, 95% CI: 1.24 – 13.51; > 2 cups OR = 5.73, 95% CI: 1.58 – 20.77). After adjusting for age, BMI and education, elevated ORs and statistical significance remained (OR = 6.56, 95% CI: 1.38 – 31.10).

Severe Menstrual Cycle Length Irregularity

In order to assess severe menstrual cycle length irregularity, the following question was asked on the retrospective questionnaire: "During the last 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills." Crude and adjusted ORs, 95% CIs, and p-values for going more than 6 weeks without a menstrual cycle and the

various markers of atrazine exposure are presented in Table 3.18. Multivariable analyses were not conducted since cell frequencies less than five were present.

A statistically significant association was observed between state of residence and going more than 6 weeks without a menstrual period (OR = 6.16; 95% CI: 1.29 – 29.38).

A frequency of zero among women going more than six weeks without a menstrual period made it impossible to conduct an only Vermont women analysis. This made it difficult to interpret associations beyond background risk. An attempt to overcome this was made by examining the ratio of cases to the number of controls. A difference in case to control ratios between the only Vermont women analysis and the Vermont and Illinois women analysis suggested an association between years in current home and going more than six weeks without a menstrual period. This was consistent with the OR which was elevated, although not significantly, (OR = 3.76, 95% CI: 0.64 – 21.97) and not in a dose response manner.

Again, an analysis among only Vermont women (background risk) could not be conducted due to no women going more than six weeks without a menstrual period. In an effort to evaluate background risk, case to control ratios for the two exposure groups were examined. Case to control ratios for unfiltered water consumption and going more than six weeks without a menstrual period among only Vermont women were similar to case to control ratios when Illinois women were included as exposed and Vermont remained as unexposed. Similar case to control ratios suggested no association was

present. The relationship between unfiltered water consumption and going more than six weeks without a menstrual period where Illinois women were considered exposed and Vermont women unexposed was not statistically significant (OR = 3.92, 95% CI: 0.60 – 25.41). In the analysis for Illinois women only and consumption of unfiltered water, no statistically significant association was found (OR = 0.50, 95% CI: 0.12 – 2.16).

Inter-Menstrual Bleeding

Inter-menstrual bleeding was assessed by the question, "During the last 12 months did you ever bleed or spot between menstrual periods (Do not count times when you were pregnant, breast feeding or using birth control pills/medication)?" Crude and adjusted ORs, 95% CIs, and p-values for inter-menstrual bleeding and the various markers of atrazine exposure are presented in Table 3.19. Overall, analyses did not reveal any statistically significant associations between any of the markers of atrazine exposure and inter-menstrual bleeding.

No significant association (OR = 1.56; 95% CI: 0.48 – 5.15) was observed between state of residence and inter-menstrual bleeding. Adjusting for vegetable consumption and income did not change the results (OR = 2.07; 95% CI: 0.61 – 7.06).

Background risk using only Vermont women in the analysis revealed no statistically significant association between years in current home and inter-menstrual bleeding (OR = 0.35; 95% CI: 0.05 – 2.29). Although ORs increased with increasing years in current home for the analysis with Vermont women as unexposed and Illinois women as

exposed, no statistically significant association was present. Compared to Vermont women, Illinois women who lived in their current home for four years or less reported similar inter-menstrual bleeding patterns (OR = 1.26; 95% CI: 0.27 – 5.76). Illinois women who lived in their homes for four years or more also reported similar inter-menstrual bleeding patterns compared to Vermont women (OR = 1.83; 95% CI: 0.48 – 6.97).

No statistically significant association was apparent between consumption of unfiltered water and inter-menstrual bleeding in the Vermont women only (background risk) analysis (OR = 1.29; 95% CI: 0.19 – 8.57). Inter-menstrual bleeding analyses comparing Illinois women drinking ≤ 2 cups/day of unfiltered water and Illinois women drinking > 2 cups/day with Vermont women were not statistically significant (≤2 cups OR = 1.26; 95% CI: 0.31 – 5.09; >2 cups OR = 2.07; 95% CI: 0.50 – 8.64).

Menstrual Cycle Length

Menstrual cycle length was assessed using the following question on the retrospective questionnaire, "Many women have their periods about once a month. Some women have their periods more often and others less often. How often are your menstrual periods? In other words, how many days are there from the first day of one menstrual period to the first day of the next period?" The categorical response choices ≤ 24 days and 25-30 days were considered not long while 31-35, 36-42 and 43 days or more were considered long. Crude and adjusted ORs, 95% CIs, and p-values for menstrual cycle length and the various markers of atrazine exposure are presented in Table 3.20. Analyses among

Illinois and Vermont women revealed no statistically significant associations between any of the markers of atrazine exposure and menstrual cycle length.

No significant association was observed between state of residence and menstrual cramps (OR = 1.43; 95% CI: 0.50 – 4.13). Statistical non-significance remained after adjusting for age and smoking (OR = 1.17; 95% CI: 0.36 – 3.79).

No statistically significant association and wide confidence intervals were observed between years in current home and menstrual cycle length in the analysis for Vermont women only (background risk) (OR = 1.67, 95% CI: 0.29 – 9.66). The magnitude of the ORs remained the same and the associations were not significant for analyses with Vermont women as unexposed and Illinois women as exposed ($\leq$ 4 years OR = 1.17, 95% CI: 0.35 – 3.97; > 4 years OR = 1.69, 95% CI: 0.57 – 5.03). Illinois women who lived in their homes for more than four years were also not more or less likely to report long menstrual cycles compared to Illinois women who lived in their homes four years or less (OR = 1.45; 95% CI: 0.40 – 5.20).

In the main analysis, with all women included, no statistically significant association was found between consumption of unfiltered water and menstrual cycle length ($\leq$2 cups OR=1.48; 95% CI: 0.50 – 4.36; >2 cups OR = 1.39; 95% CI: 0.40 – 4.79). No significant association was apparent among Illinois women only (OR = 0.94; 95% CI: 0.26 – 3.39). ORs were similar for Vermont women (background risk) (OR = 1.75; 95% CI: 0.34 – 9.05).

Menstrual Cycle Cramps

Experiencing menstrual cycle cramps was assessed by the question, "Approximately, how often do you have cramps or backache with your menstrual periods?" The categorical response choices 'never' and 'sometimes' were considered normal while 'often' and 'always' were considered not normal. Crude and adjusted ORs, 95% CIs, and p-values for menstrual cramps and the various markers of atrazine exposure are presented in Table 3.21.

No significant association was observed between state of residence and menstrual cramps (OR = 0.63; 95% CI: 0.28 – 1.42). Adjusted analyses were not conducted because confounding was determined not to be present.

In the main analysis, with all women included, no statistical significance was present between menstrual cramps and years in current home (OR = 0.92, 95% CI: 0.35 – 2.43). Among only the Vermont women, a marginally significant association was evident and the OR increased (OR = 3.43, 95% CI: 0.98 - 11.97). Among Illinois women only, the association was not statistically significant (OR = 2.55, 95% CI: 0.82 – 7.89). These associations are believed to be the consequence of random variation due to small sample size since, in general, the case to control ratios appear erratic.

In the main analysis, with all women included, no statistically significant associations were found between consumption of unfiltered water and cramps. The OR for

consumption of ≤ 2 cups/day in Illinois was 0.81, 95% CI: 0.32 – 2.05; for > 2 cups/day OR = 0.44, 95% CI: 0.16 – 1.25. A significant association between menstrual cramps and unfiltered water consumption was apparent for Vermont women (background risk) (OR = 5.83, 95% CI: 1.14 – 29.84). Again, however, it is possible these associations are the consequence of random variation due to small sample size since the case to control ratios appear erratic.

Specific Aim 7 – Atrazine exposure → diary cycle length (diary)

In order to determine whether exposure to atrazine in drinking water is associated with menstrual cycle abnormalities, menstrual cycle length was also assessed prospectively from diaries maintained by 67 women.

Mean cycle lengths, standard deviations and p-values by exposure categories are presented in Table 3.22. There were no statistically significant differences observed between mean menstrual cycle lengths for any of the atrazine exposure variables. For years in current home (Vermont as unexposed and Illinois as exposed), mean cycle lengths were greatest at the highest exposure category (> four years), although not significantly ($p = 0.30$) and not in a dose response manner. Mean cycle lengths were also greatest at the highest unfiltered water consumption exposure category (> two glasses/day) but not significantly ($p = 0.26$). Using the average of the two residential tap water samples analyzed for atrazine by CDC (dichotomized at limit of detection/$\sqrt{2}$), menstrual cycle lengths were higher with increased atrazine exposure (both with and without chlorine) but not significantly so (31.06 days compared to 30.00 days, $p = 0.62$).

There was also no significant difference in cycle length for chlorotriazine residential tap water exposure both with chlorine (27.33 days compared to 30.76 days, $p = 0.37$) and without chlorine (28.50 days compared to 31.23 days, $p = 0.09$). The only biomarker examined in urine was DEAM. The two urine samples were averaged and dichotomized at 0.36. There was no statistically significant difference between the two atrazine exposure categories and menstrual cycle length (30.46 days compared to 30.50 days, $p = 0.99$). Using results of the municipal plant monitoring (Syngenta Crop Protection, Inc.) to impute a temporally weighted average for each woman, mean menstrual cycle lengths were higher for both imputed atrazine (34.07 days compared to 30.81 days, $p = 0.55$) and imputed chlorotriazine (34.50 days compared to 29.86 days, $p = 0.34$) although not significantly higher. No significant associations were found between mean menstrual cycle length and estimated 'dose' of atrazine or chlorotriazine exposure, where estimated 'dose' was defined as the amount of unfiltered water ingested per day multiplied by the concentration of atrazine (and chlorotriazine) in drinking water. For estimated 'dose' calculated using data from the municipal plant monitoring program (Syngenta Crop Protection, Inc.) and unfiltered water consumption, mean cycle lengths were shorter for the higher exposure categories but not significantly (atrazine: 32.18 days compared to 32.67 days, $p = 0.94$; chlorotriazine: 31.63 days compared to 33.80 days, $p = 0.77$). For estimated 'dose' calculated using CDC data, mean cycle lengths were also decreased but not significantly (for both atrazine and chlorotriazine: 30.25 days compared to 30.91 days, $p = 0.78$).

Potential associations between menstrual cycle length and atrazine exposure (and markers of atrazine exposure) were also examined by linear regression. Menstrual cycle length data violated the linear regression assumptions, therefore, it was necessary to inverse square transform the data prior to analysis. Crude and adjusted β coefficients, 95% CIs, and p-values are presented in Table 3.23.

No statistically significant associations were observed between any of the atrazine exposure variables and menstrual cycle length in crude analyses and after adjusting for confounders.

Specific Aim 8 – Atrazine exposure → reproductive hormones and phase length

As an endocrine disruptor, atrazine was hypothesized to affect a woman's fertility. Previous research has shown nonconception is associated with increased follicular phase length and alterations in the following four reproductive hormones: reduced preovulatory LH, reduced mid-luteal phase $E_1$3G hormone, elevated follicular phase Pd3G hormone and reduced mid-luteal phase Pd3G hormone (150). These four hormones and follicular phase length were examined as potential precursors to an effect of atrazine on a woman's fertility. Any change in reproductive hormone secretion or follicular phase length is also capable of disrupting menstruation. In addition, the LH surge and its relationship to atrazine exposure was assessed because previous toxicological studies have shown associations between atrazine exposure and LH disruption.

Mean reproductive hormone levels, standard deviations and p-values for the difference in means by exposure categories are presented in Tables 3.24 through 3.32. Linear regression was then used in analyses evaluating the relationship between drinking water exposure to atrazine (or markers of atrazine exposure) and reproductive hormone levels. Preliminary analyses suggested that the assumptions for linear regression could be met by performing a log transformation for all of the hormones and an inverse transformation for follicular phase length. Crude β coefficients for the relationship between atrazine exposure (and markers of atrazine exposure) and log transformed hormones are presented in Tables 3.25 – 3.33. Confounding was not found to be present in any of the hormone analyses so unadjusted data are presented.

Preovulatory Luteinizing Hormone

LH is a gonadotropic hormone secreted by the anterior pituitary and the hormone in highest concentrations during the follicular phase. Pre-ovulation, it is secreted by the anterior pituitary at a fairly steady rate. Mean preovulatory LH levels, standard deviations, and p-values are presented in Table 3.24.

Similar mean preovulatory LH levels were found in Vermont and Illinois women (18.45 mIU/mg Cr compared to 15.77 mIU/mg Cr, p = 0.50). There were no significant differences observed between mean preovulatory LH level and any of the atrazine exposure variables. For years in current home (Vermont as unexposed and Illinois as exposed), mean preovulatory LH levels decreased with increased atrazine exposure, although not significantly and not in a dose response manner ($\leq$ 4 years = 18.59 mIU/mg

Cr, $p = 0.98$; > 4 years = 13.35 mIU/mg Cr, $p = 0.25$). Compared to Vermont women, mean preovulatory LH level also decreased with increasing unfiltered water consumption in Illinois women, but again not significantly nor in a dose response manner ($\leq$ 2 glasses/day = 18.53 mIU/mg Cr, $p = 0.99$; > two glasses/day = 14.04 mIU/mg Cr, $p = 0.32$). Using the average of the two residential tap water samples analyzed by CDC for atrazine, mean preovulatory LH levels decreased with increased atrazine exposure (both with and without chlorine) but not significantly so (18.45 mIU/mg Cr compared to 15.77 mIU/mg Cr, $p = 0.50$). Conversely, mean preovulatory LH levels increased with increasing chlorotriazine tap water results both with chlorine (17.28 mIU/mg Cr compared to 18.54 mIU/mg Cr, $p = 0.93$) and without chlorine (15.31 mIU/mg Cr compared to 22.96 mIU/mg Cr, $p = 0.22$). There was no statistically significant difference between levels of the biomarker DEAM and mean preovulatory LH levels (15.57 mIU/mg Cr compared to 18.43 mIU/mg Cr, $p = 0.41$). Using municipal plant monitoring data (Syngenta Crop Protection, Inc.) to impute exposure averages, mean preovulatory LH levels increased with increasing atrazine exposure (14.07 mIU/mg Cr compared to 25.08 mIU/mg Cr, $p = 0.67$) and decreased with increasing chlorotriazine exposure (17.38 mIU/mg Cr compared to 13.18 mIU/mg Cr, $p = 0.55$), although not significantly. For estimated 'dose' calculated using data from the municipal plant monitoring program (Syngenta Crop Protection, Inc.), mean preovulatory LH levels decreased with increased atrazine exposure (18.34 mIU/mg Cr compared to 14.16 mIU/mg Cr, $p = 0.55$) and for chlorotriazine (23.79 mIU/mg Cr compared to 13.36 mIU/mg Cr, $p = 0.19$). For estimated 'dose' calculated using CDC data, preovulatory LH

levels also decreased with increased atrazine and chlorotriazine exposure (for both atrazine and chlorotriazine: 19.16 mIU/mg Cr compared to 16.51 mIU/mg Cr, $p = 0.52$).

Results of linear regression analyses are presented in Table 3.25. None of the atrazine exposure markers was statistically significantly associated with preovulatory LH. Although not statistically significant, atrazine exposure appeared to be associated with small but consistent reductions in preovulatory LH concentrations across the various atrazine exposure variables.

Mid-Luteal Phase Estrone 3-Glucuronide

Mid-luteal phase $E_1$3G is a steroid hormone that (along with progesterone) stimulates endometrial growth during the luteal phase. Increased levels of estrogens during the luteal phase suppress the hypothalamic-pituitary system resulting in decreased LH and FSH.

Mean mid-luteal phase $E_1$3G levels, standard deviations, and p-values are presented in Table 3.26. Overall, mean mid-luteal phase $E_1$3G levels appeared to be reduced with increased atrazine exposure. Women living in Illinois had decreased mean mid-luteal phase $E_1$3G levels (24.15 ng/mg Cr) compared to women living in Vermont (31.34 ng/mg Cr), although the difference was not statistically significant ($p = 0.11$). Mean levels of mid-luteal phase $E_1$3G decreased in a dose response manner with increasing years in home for Illinois women (≤ 4 years = 26.59 ng/mg Cr, $p = 0.42$; > 4 years = 22.02 ng/mg Cr, $p = 0.09$) as compared to women living in Vermont (31.34 ng/mg Cr). Mean levels of

mid-luteal phase $E_1$3G also decreased in a dose response manner with increasing consumption of unfiltered water for Illinois women ($\leq$ 2 glasses/day = 24.54 ng/mg Cr, p = 0.31; > 2 glasses/day = 23.90 ng/mg Cr, p = 0.14) as compared to Vermont women (31.34 ng/mg Cr). Residential tap water levels of atrazine were also associated with a decrease in mid-luteal phase estrone 3-glucuronide level (31.34 ng/mg Cr compared to 24.15 ng/mg Cr, p = 0.11: both with chlorine and without chlorine). Mid-luteal phase $E_1$3G levels increased with increasing chlorotriazine residential tap water concentrations both with chlorine (28.15 ng/mg Cr compared to 29.49 ng/mg Cr, p = 0.87) and without chlorine (26.18 ng/mg Cr compared to 33.46 ng/mg Cr, p = 0.25) although the differences were not statistically significant. As urinary DEAM levels increased so did mean mid-luteal phase $E_1$3G levels (26.90 ng/mg Cr compared to 28.97 ng/mg Cr, p = 0.66). Using imputed municipal plant monitoring data (Syngenta Crop Protection, Inc.), mean mid-luteal phase $E_1$3G levels increased with increasing atrazine exposure (23.00 ng/mg Cr compared to 31.66 ng/mg Cr, p = 0.36) and chlorotriazine exposure (22.90 ng/mg Cr compared to 26.66 ng/mg Cr, p = 0.59). Increased estimated 'dose' of atrazine and chlorotriazine was associated with decreased mid-luteal phase $E_1$3G levels for several markers. When municipal plant (Syngenta Crop Protection, Inc.) data were used to calculate estimated 'dose', mid-luteal phase $E_1$3G levels decreased but not significantly (atrazine: 29.45 ng/mg Cr compared to 20.62 ng/mg Cr, p = 0.36; chlorotriazine: 32.99 ng/mg Cr compared to 20.94 ng/mg Cr, p = 0.09). Using CDC analyzed water samples to estimate 'dose', mid-luteal phase $E_1$3G levels were significantly decreased (both atrazine and chlorotriazine: 35.67 ng/mg Cr compared to 24.39 ng/mg Cr, p = 0.01).

Results for linear regression analyses of mid-luteal phase $E_1$3G are presented in Table 3.27. A suggestive association (β = -0.32; 95% CI: -0.68 – 0.04) was observed between state of residence and urinary mid-luteal phase $E_1$3G. An imprecise association was also observed between living in current home more than four years and mid-luteal phase $E_1$3G (β = -0.43; 95% CI: -0.87 – 0.02), although this association was not apparent in the analysis of Illinois women only (β = -0.23; 95% CI: -0.93 – 0.46). Residential tap water results both with and without chlorine suggested an association with mid-luteal phase $E_1$3G (β = -0.32; 95% CI: -0.68 – 0.04, for both atrazine and chlorotriazine). Furthermore, when consumption of water was included in the exposure calculation (i.e., estimated 'dose'), the association was strengthened and became statistically significant for both atrazine and chlortriazine (β = -0.46; 95% CI: -0.82 – -0.10, both atrazine and chlorotriazine).

Mid-Luteal Phase Pregnanediol 3-Glucuronide

Mid-luteal phase Pd3G is a steroid hormone and the dominant hormone of the luteal phase. Increased levels of progesterone during the luteal phase ensue in decreased LH and FSH. Near the end of the luteal phase, progesterone levels decrease resulting in endometrium disintegration and menstruation.

Mean mid-luteal phase Pd3G levels, standard deviations and p-values are presented in Table 3.28. Overall, mid-luteal phase Pd3G appeared to decrease with increasing atrazine exposure. Women living in Illinois had lower mid-luteal phase Pd3G levels

(9.73 μg/mg Cr) compared to women living in Vermont (10.93 μg/mg Cr), but the difference was not statistically significant different ($p = 0.51$). With increasing years in current home (Vermont as unexposed and Illinois as exposed), mean mid-luteal phase Pd3G decreased, although not significantly and not in a dose response manner (Vermont 10.93 μg/mg Cr; ≤ 4 years = 11.55 μg/mg Cr, $p = 0.81$; > 4 years = 8.13 μg/mg Cr, $p = 0.23$). Compared to Vermont women, mean mid-luteal phase Pd3G hormone levels also decreased with increasing unfiltered water consumption in Illinois women, but not significantly nor in a dose response manner (Vermont = 10.93 μg/mg Cr; ≤ 2 glasses/day = 11.24 μg/mg Cr, $p = 0.91$; > two glasses/day = 8.72 μg/mg Cr, $p = 0.34$). As atrazine concentrations in tap water increased, mean mid-luteal phase Pd3G levels decreased (both with chlorine and without chlorine: 10.93 μg/mg Cr compared to 9.73 μg/mg Cr, $p = 0.51$). Conversely, increasing chlorotriazine levels resulted in decreased, non-significant mean mid-luteal phase Pd3G levels (with chlorine: 10.39 μg/mg Cr compared to 10.63 μg/mg Cr, $p = 0.94$; without chlorine: 9.50 μg/mg Cr compared to 12.70 μg/mg Cr, $p = 0.11$). There was no statistically significant difference between the biomarker DEAM and mean mid-luteal phase Pd3G levels (9.84 μg/mg Cr compared to 10.72 μg/mg Cr, $p = 0.65$). There was also no statistically significant difference between either imputed atrazine or imputed chlorotriazine concentration (using municipal plant monitoring data by Syngenta Crop Protection, Inc.) and mean mid-luteal phase Pd3G levels (atrazine: 9.87 μg/mg Cr compared to 8.78 μg/mg Cr, $p = 0.74$; chlorotriazine: 9.11 μg/mg Cr compared to 10.97 μg/mg Cr, $p = 0.44$). For estimated 'dose' calculated using data from the municipal plant monitoring program (Syngenta Crop Protection, Inc.), mean mid-luteal phase Pd3G levels decreased with increasing atrazine (7.92 μg/mg

Cr compared to 12.44 μg/mg Cr, p = 0.02) and chlorotriazine (12.08 μg/mg Cr compared to 8.87 μg/mg Cr, p = 0.20). For estimated 'dose' calculated using CDC water data, mean mid-luteal phase Pd3G levels decreased with increasing atrazine and chlorotriazine (for both atrazine and chlorotriazine: 11.94 μg/mg Cr compared to 9.62 μg/mg Cr, p = 0.22).

Results of the mid-luteal phase pregnanediol linear regression analyses are presented in Table 3.29. Mid-luteal phase Pd3G was significantly associated with atrazine estimated 'dose' using municipal plant data (Syngenta Crop Protection, Inc.) (β = -0.57; 95% CI: -1.06 – -0.09). Imprecise associations were also present with estimated 'dose' chlorotriazine (β = -0.43; 95% CI: -1.03 – 0.18). When CDC analyzed water samples were used to estimate 'dose', imprecise associations were also present for both atrazine and chlorotriazine exposure (β = -0.31; 95% CI: -0.71 – 0.08, for both atrazine and chlorotriazine).

Luteinizing Hormone Surge

The LH surge is the mid-point of the menstrual cycle initiating ovulation. The LH surge is the transition point from the follicular to the luteal phase of the cycle and the endpoint found to be affected in laboratory animal studies with atrazine.

Mean LH levels, standard deviation and p-values are presented in Table 3.30. Overall, mean LH levels decreased with increasing atrazine exposure. There were no statistically significant differences observed between mean LH surge level and state of residence

(Vermont = 55.02 mIU/mg Cr compared to Illinois 49.27 mIU/mg Cr, $p = 0.42$). For years in current home (Vermont as unexposed and Illinois as exposed), the mean LH surge level decreased with increased atrazine exposure, although not significantly and not in a dose response manner (Vermont = 55.02 mIU/mg Cr; ≤ 4 years = 45.22 mIU/mg Cr, $p = 0.31$; > 4 years = 52.31 mIU/mg Cr, $p = 0.75$). Compared to Vermont women, mean LH level decreased with increasing unfiltered water consumption in Illinois women, and although not significantly, a dose response pattern was observed (Vermont = 55.02 mIU/mg Cr; ≤ 2 glasses/day = 49.70 mIU/mg Cr, $p = 0.60$; > two glasses/day = 48.94 mIU/mg Cr, $p = 0.45$). Mean LH surge levels decreased with increased atrazine exposure as measured in residential tap water, but not significantly (both with and without chlorine = 55.02 mIU/mg Cr compared to 49.27 mIU/mg Cr, $p = 0.42$). The relationship between the LH surge and chlorotriazine tap water exposure was conflicting between water samples with and without chlorine, with hormone levels of samples with chlorine decreasing and hormone levels of those without chlorine increasing (with chlorine = 53.88 mIU/mg Cr compared to 39.63 mIU/mg Cr, $p = 0.25$; without chlorine = 46.37 mIU/mg Cr compared to 66.86 mIU/mg Cr, $p = 0.005$). There was no statistically significant difference between the DEAM and mean LH surge levels (50.87 mIU/mg Cr compared to 53.56 mIU/mg Cr, $p = 0.72$). Using municipal plant monitoring data (Syngenta Crop Protection, Inc.) to impute exposure averages, mean LH surge levels increased significantly with increasing atrazine exposure (44.53 mIU/mg Cr compared to 77.69 mIU/mg Cr, $p = 0.03$) and decreased with increasing chlorotriazine exposure (49.82 mIU/mg Cr compared to 48.27 mIU/mg Cr, $p = 0.90$), although not significantly. For estimated 'dose' calculated using data from the municipal water plant monitoring

program (Syngenta Crop Protection, Inc.), mean LH levels decreased with increased atrazine exposure (51.11 mIU/mg Cr compared to 47.89 mIU/mg Cr, $p = 0.79$) as well as with increased chlorotriazine exposure (63.34 mIU/mg Cr compared to 43.64 mIU/mg Cr, $p = 0.11$). For estimated 'dose' calculated using CDC analyzed water data, LH surge levels also decreased with increased atrazine and chlorotriazine exposure but the differences were not significant (for both atrazine and chlorotriazine: 55.99 mIU/mg Cr compared to 50.87 mIU/mg Cr, $p = 0.50$).

Results of the linear regression analyses for LH surge are presented in Table 3.31. The positive findings for LH surge were limited to statistically significant associations with chlorotriazine tap water concentrations both with chlorine (β = -0.48; 95% CI: -0.97 – 0.00) and without chlorine (β = 0.39; 95% CI: 0.10 – 0.68). However, due to small numbers the fact that the associations were in opposite directions, interpretation of this analysis is uncertain. No other statistically significant associations between atrazine exposure and LH peak were present.

Follicular Pregnanediol 3-Glucuronide

Pd3G is secreted in small amounts during the follicular phase. A rise in progesterone prompts the FSH surge.

Mean follicular phase Pd3G levels, standard deviations, and p-values are presented in Table 3.32. Overall, there were no significant differences between atrazine exposures (or markers of atrazine exposure) and follicular phase Pd3G mean concentrations. Similar

mean follicular phase Pd3G concentrations were measured in women residing in Illinois compared to women residing in Vermont (0.98 μg/mg Cr compared to 0.82 μg/mg Cr, p = 0.51). Mean follicular phase Pd3G concentrations decreased with increasing years in current home (Vermont as unexposed and Illinois as exposed), although not significantly and not in a dose response manner (Vermont 0.98 μg/mg Cr; ≤ 4 years = 1.01 μg/mg Cr, p = 0.92; > 4 years = 0.66 μg/mg Cr, p = 0.18). Compared to Vermont women, mean follicular phase Pd3G hormone levels also decreased with increasing unfiltered water consumption in Illinois women, but, again, not significantly nor in a dose response manner (Vermont = 0.98 μg/mg Cr; ≤ 2 glasses/day = 0.68 μg/mg Cr, p = 0.21; > two glasses/day = 0.91 μg/mg Cr, p = 0.80). Follicular phase Pd3G mean levels were non-significantly decreased with increasing atrazine tap water concentrations (both with chlorine and without chlorine: 0.98 μg/mg Cr compared to 0.82 μg/mg Cr, p = 0.51). Follicular phase Pd3G levels were also non-significantly decreased with increasing chlorotriazine tap water concentrations (with chlorine: 0.93 μg/mg Cr compared to 0.71 μg/mg Cr, p = 0.65; without chlorine: 0.95 μg/mg Cr compared to 0.81 μg/mg Cr, p = 0.50). Mean hormone levels increased, although not statistically significantly, with increasing DEAM levels in urine (0.71 μg/mg Cr compared to 1.02 μg/mg Cr, p = 0.14). Using municipal plant monitoring data (Syngenta Crop Protection, Inc.) to impute exposure concentrations, there was no statistically significant difference between either imputed atrazine or imputed chlorotriazine concentration and mean follicular phase Pd3G levels (atrazine: 0.86 μg/mg Cr compared to 0.57 μg/mg Cr, p = 0.31; chlorotriazine: 0.82 μg/mg Cr compared to 0.83 μg/mg Cr, p = 0.95). There were also no statistically significant differences between mean follicular phase Pd3G levels and atrazine estimated

'dose' calculated using data from the municipal plant monitoring program (Syngenta Crop Protection, Inc.) (0.72 μg/mg Cr compared to 0.89 μg/mg Cr, p = 0.32) or chlorotriazine estimated 'dose' (0.67 μg/mg Cr compared to 0.88 μg/mg Cr, p = 0.33). Again, no statistically significant differences existed between mean follicular phase Pd3G levels and either estimated 'dose' (atrazine or chlorotriazine) calculated using CDC water data (both atrazine and chlorotriazine = 1.14 μg/mg Cr compared to 0.79 μg/mg Cr, p = 0.36).

Results of linear regression analyses are presented in Table 3.33. There was little evidence of any association between follicular phase Pd3G and any of the atrazine exposure variables. Variability was observed both in the magnitude and direction of the β coefficients.

Follicular Phase Length

The follicular phase is the most variable of the two phases of the menstrual cycle and the time when the follicles in the ovary mature. It begins with menstruation and ends with ovulation.

Follicular phase length means, standard deviations, and p-values of differences in means by exposure category are presented in Table 3.34. Data for follicular phase length were subjected to inverse transformation to satisfy the assumptions of the linear regression model. Crude and adjusted β coefficients for the relationship between atrazine exposure

(and markers of atrazine exposure) and inverse transformed follicular phase length are presented in Table 3.35.

Mean follicular phase length, standard deviation, and p-values are presented in Table 3.34. No statistically significant difference was observed between any of the atrazine exposures (or markers of exposure) and mean follicular phase length. In addition, follicular phase length both increased and decreased with increasing atrazine exposure. There was little difference between mean follicular phase length and state of residence (16.00 days compared to 15.87 days, $p = 0.94$). There was no statistically significant difference between years in current home (Vermont as unexposed and Illinois as exposed), and follicular phase length (Vermont 16.00 days; ≤ 4 years = 14.00 days, $p = 0.38$; > 4 years = 17.50 days, $p = 0.52$). There was also no statistically significant difference between unfiltered water consumption and follicular phase length (Vermont = 16.00 days; ≤ 2 glasses/day = 14.00 days, $p = 0.41$; > two glasses/day = 17.10 days, $p = 0.61$). Follicular phase length decreased with increased atrazine exposure measured via residential tap water samples both with and without chlorine, although not significantly (for both with chlorine and without = 16.00 days compared to 15.87 days, $p = 0.94$). There was also no statistically significant difference in follicular phase length for chlorotriazine residential tap water exposure both with (16.16 days compared to 13.67 days, $p = 0.43$) and without chlorine (16.40 days compared to 14.80 days, $p = 0.28$). Follicular phase lengths were statistically similar between increasing DEAM and follicular phase length (16.17 days compared to 15.83 days, $p = 0.86$). Using results of the municipal plant monitoring program (Syngenta Crop Protection, Inc.) to impute a

temporally weighted average for each woman, mean follicular phase lengths were shorter for increasing atrazine exposure (16.08 days compared to 14.50 days, p = 0.68) and longer for increasing chlorotriazine (14.30 days compared to 19.00 days, p = 0.19) although neither was significantly different. For estimated 'dose' calculated using data from the municipal plant monitoring program (Syngenta Crop Protection, Inc.), follicular phase lengths were longer for the higher exposure categories (atrazine = 13.83 days compared to 17.22 days, p = 0.19; chlorotriazine = 14.00 days compared to 16.55, p = 0.38). However, for estimated 'dose' calculated using CDC data, follicular phase lengths decreased (atrazine and chlorotriazine = 16.92 days compared to 15.43 days, p = 0.51).

Results of linear regression analyses of follicular phase length analyses are presented in Table 3.35. Parity was consistently identified as a confounder in each of the follicular phase length analyses and therefore, included in all of the analyses. Other confounders were evaluated as well; however, none changed the overall interpretation (statistical significance or β coefficient magnitude). Therefore, for efficiency of the model, additional confounders were not included. An imprecise association between municipal plant chlorotriazine monitoring data (Syngenta Crop Protection, Inc.) and follicular phase length was present (β = -0.016; 95% CI: -0.03 – 0.00). This association became statistically significant with adjustment for parity (β = -0.019; 95% CI: -0.04 – 0.00). When municipal plant data (Syngenta Crop Protection, Inc.) were used to calculate estimated 'dose', follicular phase length was statistically significantly associated with atrazine estimated 'dose' (β = -0.021; 95% CI: -0.04 – 0.00) and chlorotriazine estimated 'dose' (β = -0.023; 95% CI: -0.04 – 0.00). These associations remained significant after

adjusting for parity. No other statistically significant associations were present; however, these analyses were based on a small number of exposed women.

Specific Aim 9 – Phase length and Pd3G → menstrual cycle characteristics

Associations between the independent variables (follicular phase length, luteal phase length and peak Pd3G concentr ations) and the dependent variables (menstrual cycle length, menstrual cycle length irregularity, and menstrual cycle severe length irregularity) are presented in Tables 3.36-3.38. Studies have shown follicular phase length contributes most to the variability of the menstrual cycle (200) while the luteal phase length is comparatively stable and consistent (201). Peak progesterone concentrations are also highly correlated with menstrual cycle characteristics (J Kesner, personal communication, May 2008). The purpose of this analysis was to attempt to confirm these relationships in order to test the sensitivity of the methods used to evaluate potential effects of atrazine exposure on reproductive endocrinology. Therefore, it was expected that follicular phase length and peak Pd3G concentrations would be associated with menstrual cycle characteristics. However, it was not expected that luteal phase length would be associated with menstrual cycle characteristics.

As described previously, length irregularity was assessed by the question, "Generally speaking, are your periods [cycles] regular or irregular? That is, is the length of time between the first day of one period and the first day of the other about the same each cycle?" Severe length irregularity (i.e., going more than six weeks without a menstrual period) was assessed by the question, "During the last 12 months, did you ever go for

more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills." Menstrual cycle length (in days) was reported by the prospective menstrual cycle diary and examined as a continuous variable.

Crude β coefficients for the relationship between the independent variables (luteal phase length, follicular phase length and peak Pd3G) and total menstrual cycle length (as reported by prospective menstrual cycle diary) are presented in Table 3.36. Adjusted β coefficients were not calculated because none of the covariates were determined to be acting as confounders. Significant associations were observed between follicular phase length and menstrual cycle length (β = 0.93; 95% CI: 0.82 – 1.04). There was no evidence of an association between peak Pd3G and menstrual cycle length (β = -0.09; 95% CI: -0.39 – 0.21). As expected, there was no association between luteal phase length and menstrual cycle length (β = 0.05; 95% CI: -1.08 – 1.19).

Crude ORs for the relationship between the independent variables (luteal phase length, follicular phase length and peak Pd3G) and length irregularity are presented in Table 3.37. Crude ORs for the relationship between the independent variables (luteal phase length, follicular phase length and peak Pd3G) and severe length irregularity (i.e. going more than six weeks without a menstrual period) are presented in Table 3.38. Confounding was not assessed for either outcome (length irregularity or severe length irregularity) since the number of cases (for both length irregularity and severe length irregularity) was less than five.

There was little evidence of an association between any of the independent variables (luteal phase length, follicular phase length and peak Pd3G) and menstrual cycle length irregularity. Although, the association between follicular phase length and length irregularity was the closest to statistical significance (OR = 1.10; 95% CI: 0.93 – 1.31).

A marginally statistically significant protective association was present between follicular phase length and severe length irregularity (OR = 0.33; 95% CI: 0.11 – 1.00) (Table 3.38). Although not statistically significant, an increase in luteal phase length was apparent among women with severe length irregularity (95% CI: 0.91 – 15.59). There was no association between peak Pd3G and severe length irregularity (β = 1.05; 95% CI: 0.82 – 1.34). However, conclusions should not be drawn from these data (length irregularity and severe length irregularity) since the results are based on three and two cases for length irregularity and severe length irregularity, respectively.

Specific Aim 10 – Comparison of retrospective and prospective menstrual cycle characteristics

The goal of this analysis was to compare retrospectively reported menstrual cycle characteristics with prospectively maintained menstrual cycle diaries in an effort to investigate the reliability of questionnaire-based categorical menstrual cycle length data.

A total of 67 women, 18 to 40 years old residing in Mount Olive and Gillespie, Illinois and Waterbury and Fair Haven, Vermont first answered a retrospective questionnaire

then completed a prospective menstrual cycle diary. Women who answered the questionnaire and maintained the menstrual cycle diary were predominantly white, without a four year college degree but with some college education and with a family income of less than $60,000 per year (Table 3.39). They ranged in age from 18 to 40, with a mean of 33 years (standard deviation = 5.6). The mean BMI was 26.2 (standard deviation = 5.69) (Table 3.39).

The association between demographic characteristics (age, BMI, education, income, alcohol, smoking, and physical activity) and agreement between diary and questionnaire responses was assessed and presented in Table 3.39. There were no statistically significant differences in age, BMI, education, income, alcohol, smoking, or physical activity between the concordant and discordant groups. The effect of menstrual cycle length regularity on agreement between diary and questionnaire responses was also evaluated and presented in Table 3.39. Women whose prospective and retrospective data were concordant were more likely to report normal cycle length.

Since it was uncertain how a regular menstrual cycle length should be defined, two different definitions were used, 25-30 days (definition 1) and 25-35 days (definition 2). According to definition 1 (25-30 days), 18% (12/67) of women reported having a regular cycle on both the questionnaire and the diary and 51% (34/67) of women reported not having a regular cycle on both the questionnaire and the diary (Table 3.40). This resulted in 69% (46/67) overall agreement for definition 1. According to definition 2 (25-35 days), 9% (6/67) reported a normal cycle on both the questionnaire and the diary and

75% (50/67) reported not having a normal cycle on both the questionnaire and the diary which resulted in 83.6% (56/67) overall agreement (Table 3.40).

The analysis of agreement (Cohen's kappa) for regularity resulted in modest agreement for both definition 1 (25-30 days) (kappa=0.31) and definition 2 (25-35 days) (kappa=0.43) (Table 3.40). The prevalence indices were -0.33 for definition 1 and -0.66 for definition 2.

Table 3.1. Demographic and potential confounding characteristics of the study population; total population and stratified by state

	TOTAL		*Vermont*		*Illinois*	
Characteristics	***N=102***	***Percent***	***N=49***	***Percent***	***N=53***	***Percent***
Age (years)						
18-34	52	50.98	21	42.86	31	58.49
35-40	50	49.02	28	57.14	22	41.51
Age (years)						
18-23	5	4.90	0	0.00	5	9.43
24-29	11	10.78	4	8.16	7	13.21
30-35	43	42.16	21	42.86	22	41.51
36-40	43	42.16	24	48.98	19	35.85
Age (years)						
Mean (SD)	33.79 (5.02)		35.06 (3.93)		32.62 (5.63)	
Body Mass Index						
<25	47	46.53	25	52.08	22	41.51
≥25	54	53.47	23	47.92	31	58.49
Body Mass Index						
Mean (SD)	26.33 (5.86)		25.37 (4.61)		27.21 (6.72)	
Race						
White	101	99.02	49	100	52	98.11
Other	1	0.98	0	0.00	1	1.89
Hispanic						
No	101	99.02	49	100	52	98.11
Yes	1	0.98	0	0.00	1	1.89
Education						
Not College Graduate	62	60.78	25	51.02	37	69.81
College Graduate	40	39.22	24	48.98	16	30.19
Income						
< $60,000	64	67.37	31	64.58	33	70.21
≥ $60,000	31	32.63	17	35.42	14	29.79
Income						
<$15,000	4	4.08	0	0.00	4	8.00
$15,000 - $29,999	9	9.18	2	4.17	7	14.00
$30,000 - $44,999	20	20.41	9	18.75	11	22.00
$45,000 - $59,999	31	31.63	20	41.67	11	22.00
$60,000 - $74,999	11	11.22	7	14.58	4	8.00
$75,000 - $99,999	8	8.16	3	6.25	5	10.00
$100,000 - $114,999	3	3.06	2	4.17	1	2.00
$115,000 - $129,999	4	4.08	3	6.25	1	2.00
Over $130,000	5	5.10	2	4.17	3	6.00
Refuse	3	3.06	0	0.00	3	6.00

Table 3.1. continued

	TOTAL		Vermont		Illinois	
Characteristics	***N=102***	***Percent***	***N=49***	***Percent***	***N=53***	***Percent***
Current Smoker						
No	77	75.49	40	81.63	37	69.81
Yes	25	24.51	9	18.37	16	30.19
Alcohol Consumption (times per week)						
< 1	56	54.90	23	46.94	33	62.26
≥ 1	46	45.10	26	53.06	20	37.74
Caffeine Consumption (mg per day)						
< 300	81	79.41	40	75.47	41	83.67
≥ 300	21	20.59	13	24.53	8	16.33
Vegetables (servings per day)						
≤ 1	38	37.25	17	34.69	21	39.62
> 1	64	62.75	32	65.31	32	60.38
Fruit (servings per day)						
≤ 1	63	61.76	30	61.22	33	62.26
> 1	39	38.24	19	38.78	20	37.74
Multi-vitamin						
Yes	34	33.33	22	44.9	12	22.64
No	68	66.67	27	55.1	41	77.36
Weekly Physical Activity (hours)						
0-3	51	50.00	23	46.94	28	52.83
> 3	51	50.00	26	53.06	25	47.17

SD, standard deviation.

Table 3.2. Reproductive characteristics of the study population; total population and stratified by state

	TOTAL		*Vermont*		*Illinois*	
Characteristics	***N=102***	***Percent***	***N=49***	***Percent***	***N=53***	***Percent***
Infertile						
Conceived in < 1 year	60	71.43	33	76.74	27	68.85
Tried > 1 year	24	28.57	10	23.26	14	34.15
Number of pregnancies						
0	17	16.67	6	12.24	11	20.75
1-2	44	43.14	27	55.1	17	32.08
≥ 3	41	40.2	16	32.65	25	47.17
Number of Pregnancies						
Mean (SD)	2.27 (1.62)		2.12 (1.52)		2.42 (1.71)	
Number of Live Births						
0	22	21.57	9	18.37	13	24.53
1-2	52	50.98	31	63.27	21	39.62
≥ 3	28	27.45	9	18.37	19	35.85
Number of Live Births						
Mean (SD)	1.70 (1.17)		1.59 (1.04)		1.79 (1.28)	

SD, standard deviation.

Table 3.3. Mean, standard deviation, range and frequency of samples below, at, and above the limit of detection for water samples with chlorine

	N	Mean (ppb)	SD	Range (ppb)	<LOD	LOD	>LOD
Day 1							
Atrazine	39	0.58	0.38	0.03 – 1.83	2	22	15
Chlorotriazine	39	2.44	0.71	1.76 – 6.12	-	-	-
Diaminochlorotriazine	39	0.54	0.27	0.15 – 1.12	16	20	3
Desisopropylatrazine	39	0.62	0.20	0.30 – 1.37	13	25	1
Desethylatrazine	39	0.70	0.21	0.28 – 1.80	12	24	3
Atrazine mercapturate	39	0.35	0.0	0.35 – 0.35	0	39	0
Day 2							
Atrazine	39	0.65	0.38	0.35 – 1.91	0	21	18
Chlorotriazine	39	2.46	0.66	1.38 – 5.42	-	-	-
Diaminochlorotriazine	39	0.54	0.30	0.15 – 1.63	21	15	3
Desisopropylatrazine	39	0.58	0.19	0.19 – 1.07	17	21	1
Desethylatrazine	39	0.70	0.20	0.15 – 1.56	10	21	8
Atrazine mercapturate	39	0.35	0.0	0.35 – 0.35	0	39	0

LOD, limit of detection; SD, standard deviation

Table 3.4. Mean, standard deviation, range and frequency of samples below, at, and above the limit of detection for water samples without chlorine

	N	Mean (ppb)	SD	Range (ppb)	<LOD	LOD	>LOD
Day 1							
Atrazine	39	0.52	0.27	0.04 – 1.43	1	21	17
Chlorotriazine	39	3.09	2.76	1.27 – 17.74	-	-	-
Diaminochlorotriazine	39	1.08	1.99	0.15 – 11.74	18	13	8
Desisopropylatrazine	39	0.56	0.20	0.16 – 0.71	14	25	0
Desethylatrazine	39	0.92	0.78	0.20 – 4.93	11	17	11
Atrazine mercapturate	39	0.35	0.0	0.35 – 0.35	0	39	0
Day 2							
Atrazine	39	0.54	0.25	0.35 – 1.34	0	21	18
Chlorotriazine	39	2.71	2.04	1.48 – 14.49	-	-	-
Diaminochlorotriazine	39	0.82	1.44	0.16 – 9.18	20	13	6
Desisopropylatrazine	39	0.59	0.19	0.20 – 0.71	12	27	0
Desethylatrazine	39	0.76	0.62	0.17 – 4.25	14	17	8
Atrazine mercapturate	39	0.35	0.0	0.35 – 0.35	0	39	0

LOD, limit of detection; SD, standard deviation

Table 3.5. Spearman correlation coefficients and p-values for residential tap water (atrazine and chlorotriazine) and urine (desethylatrazine mercaptureate).

	N	Spearman correlation coefficient	p-value
Water – Atrazine			
Day 1 vs. Day 2 (with chlorine)	39	0.81	<0.0001
Day 1 vs. Day 2 (without chlorine)	39	0.78	<0.0001
Chlorine vs. Non-Chlorine (Day 1)	39	0.87	<0.0001
Chlorine vs. Non-Chlorine (Day 2)	39	0.95	<0.0001
Water - Chlorotriazine			
Day 1 vs. Day 2 (with chlorine)	39	0.32	0.04
Day 1 vs. Day 2 (without chlorine)	39	0.49	0.002
Chlorine vs. Non-Chlorine (Day 1)	39	0.52	0.001
Chlorine vs. Non-Chlorine (Day 2)	39	0.35	0.03
Urine - Desethylatrazine mercaptureate			
Day 1 vs. Day 2	39	0.23	0.16

Table 3.6. Atrazine and chlorotriazine tap water concentrations; total population and stratified by state

Contaminant	TOTAL Mean (SD)	Vermont Mean (SD)	Illinois Mean (SD)	p-value*
Atrazine (ppb) (with chlorine)	0.61 (0.36)	0.35 (0.04)	0.93 (0.31)	<0.001
Atrazine (ppb) (without chlorine)	0.53 (0.24)	0.35 (0.0)	0.74 (0.22)	<0.001
Chlorotriazine (ppb) (with chlorine)	2.45 (0.65)	2.32 (0.22)	2.60 (0.91)	0.22
Chlorotriazine (ppb) (without chlorine)	2.90 (2.33)	3.25 (3.10)	2.48 (0.72)	0.28

SD, standard deviation

* p-value comparing mean atrazine (or chlorotriazine) levels between Vermont and Illinois.

Table 3.7. Syngenta community water system atrazine monitoring for Mount Olive, Illinois.

Sample Date	Finished Water Atrazine (ppb)
1/3/05	0.77
1/18/05	0.47
1/31/05	0.33
1/31/05*	**
2/14/05	0.24
3/2/05	0.17
3/14/05	0.15
3/28/05	0.16
4/4/05	0.16
4/11/05	0.15
4/18/05	0.17
4/25/05	0.19
5/2/05	0.24
5/9/05	0.27
5/16/05	1.17
5/23/05*	**
5/24/05	0.05
5/31/05	0.26
6/6/05	0.30
6/13/05	0.23
6/20/05	0.24
6/27/05	0.20
7/5/05	0.15
7/11/05	**0.11**
7/18/05	**0.21**
7/25/05	**0.05**
8/1/05	**0.18**
8/8/05*	**
8/15/05	**0.12**
8/29/05	**0.20**
9/12/05	**0.21**
9/26/05	0.16
10/11/05	0.12
10/24/05	0.05
10/24/05*	**
11/7/05	0.05
11/21/05	0.05
12/05/05	0.13
12/19/05	0.10

* Illinois EPA Drinking Water Watch quarterly testing results.

** Indicates a non-detection or concentration below the limit of detection.

Note: Bolding indicates study time period.

Table 3.8. Syngenta community water system chlorotriazine monitoring for Mount Olive, Illinois.

Sample Date	Finished Water Chlorotriazine (ppb)
1/03/05	1.25
1/18/05	0.77
1/31/05	0.58
2/14/05	0.45
3/02/05	0.32
3/14/05	0.30
3/28/05	0.31
4/04/05	0.31
4/11/05	0.30
4/18/05	0.32
4/25/05	0.34
5/02/05	0.39
5/09/05	0.42
5/16/05	1.86
5/24/05	0.20
5/31/05	0.46
6/06/05	0.53
6/13/05	0.47
6/20/05	0.39
6/27/05	0.55
7/05/05	0.50
7/11/05	**0.46**
7/18/05	**0.42**
7/25/05	**0.40**
8/01/05	**0.60**
8/15/05	**0.47**
8/29/05	**0.63**
9/12/05	**0.65**
9/26/05	0.61
10/11/05	0.47
10/24/05	0.40
11/07/05	0.40
11/21/05	0.40
12/05/05	0.48
12/19/05	0.45

Note: Bolding indicates study time period.

Table 3.9. Syngenta community water system atrazine monitoring for Gillespie, Illinois.

Sample Date	Finished Water Atrazine (ppb)
1/03/05	1.17
1/19/05	0.14
1/31/05	0.16
2/14/05	0.15
2/16/05*	**
2/28/05	0.05
3/14/05	0.13
3/28/05	0.05
4/04/05	0.20
4/12/05	0.27
4/18/05	0.22
4/20/05*	**
4/25/05	0.33
5/02/05	0.18
5/09/05	0.05
5/16/05	0.87
5/23/05	0.32
5/31/05	0.50
6/06/05	0.44
6/13/05	0.65
6/20/05	0.26
6/27/05	0.56
7/05/05	0.38
7/11/05	**0.61**
7/18/05	**0.44**
7/25/05	**0.25**
8/01/05	**0.60**
8/15/05	**0.25**
8/16/05*	**
8/29/05	**0.27**
9/12/05	**0.36**
9/26/05	0.30
10/11/05	0.20
10/24/05	0.05
11/07/05	0.05
11/16/05*	**
11/21/05	0.29
12/05/05	0.17
12/19/05	0.11

* Illinois EPA Drinking Water Watch quarterly testing results.

** Indicates a non-detection or concentration below the limit of detection.

Note: Bolding indicates study time period.

Table 3.10. Syngenta community water system chlorotriazine monitoring for Gillespie, Illinois.

Sample Date	Finished Water Chlorotriazine (ppb)
1/03/05	2.02
1/19/05	0.44
1/31/05	0.47
2/14/05	0.75
2/28/05	0.45
3/14/05	0.66
3/28/05	0.40
4/04/05	0.39
4/12/05	0.48
4/18/05	0.41
4/25/05	0.57
5/2/05	0.44
5/09/05	0.26
5/16/05	2.08
5/23/05	0.55
5/31/05	0.80
6/06/05	0.72
6/13/05	1.01
6/20/05	0.69
6/27/05	0.89
7/05/05	0.77
7/11/05	**1.16**
7/18/05	**1.06**
7/25/05	**0.72**
8/01/05	**1.15**
8/15/05	**0.85**
8/29/05	**0.86**
9/12/05	**1.11**
9/26/05	0.94
10/11/05	0.65
10/24/05	0.40
11/7/05	0.40
11/21/05	0.64
12/05/05	0.52
12/19/05	0.46

Note: Bolding indicates study time period.

Table 3.11 Spearman correlation coefficients for atrazine and chlorotriazine water analyses of Illinois women between residential tap water samples (as analyzed by CDC) and imputed* municipal plant samples (as analyzed by Syngenta Crop Protection, Inc.).

	N	Spearman correlation coefficient	p-value
Atrazine (residential) vs. Imputed Atrazine (municipal)	39	0.04	0.89
Chlorotriazine (residential) vs. Imputed Chlorotriazine (municipal)	39	0.18	0.51

* The two municipal plant (Syngenta Crop Protection, Inc.) results closest in time to each woman's date of participation averaged and weighted according to their distance from the woman's participation date.

Table 3.12. Mean, standard deviation, range and frequency of samples below, at, and above the limit of detection for urine samples.

	N	Mean (ppb)	SD	Range (ppb)	<LOD	LOD	>LOD
Day 1							
Atrazine	39	0.36	0.02	0.35 – 0.51	0	38	1
Diaminochlorotriazine	39	0.63	0.14	0.25 – 0.88	12	26	1
Desisopropylatrazine	39	0.92	0.54	0.71 – 2.72	0	33	6
Desethylatrazine	39	0.84	0.65	0.71 – 4.73	0	36	3
Atrazine mercapturate	39	0.35	0.0	0.35 – 0.35	0	39	0
Desethylatrazine mercaptureate	39	11.94	44.90	0.35 – 263.51	0	21	18
Day 2							
Atrazine	39	0.45	0.44	0.35 – 2.72	0	37	2
Diaminochlorotriazine	39	0.69	0.13	0.32 – 1.22	5	33	1
Desisopropylatrazine	39	1.02	0.88	0.71 – 4.61	0	33	6
Desethylatrazine	39	0.76	0.25	0.59 – 2.34	1	35	3
Atrazine mercapturate	39	0.35	0.0	0.35 – 0.35	0	39	0
Desethylatrazine mercaptureate	39	10.90	33.15	0.35 – 153.98	0	22	17

LOD, limit of detection; SD, standard deviation

Table 3.13. Urinary desethylatrazine mercaptureate concentrations; total population and stratified by state

Contaminant	TOTAL Mean (SD)	Vermont Mean (SD)	Illinois Mean (SD)	p-value*
Desethylatrazine mercaptureate (ppb)	11.42 (34.92)	14.44 (41.50)	7.89 (25.99)	0.57

SD, standard deviation

* p-value comparing mean desethylatrazine mercaptureate levels between Vermont and Illinois

Table 3.14. Years in home, water consumption and atrazine dose exposure characteristics; total population and stratified by state

	TOTAL		*Vermont*		*Illinois*	
Characteristics	***N=102***	***Percent***	***N=49***	***Percent***	***N=53***	***Percent***
Years in Current Home						
< 4	42	41.18	18	36.73	24	45.28
≥ 4	60	58.82	31	63.27	29	54.72
Years in Current Home						
Mean (SD)	6.40 (4.89)		6.12 (3.99)		6.66 (5.62)	
Total Water Consumption (cups per day)						
≤ 8	60	58.82	24	48.98	36	67.92
> 8	42	41.18	25	51.02	17	32.08
Total Water Consumption						
Mean (SD)	7.86 (4.32)		8.20 (4.25)		7.54 (4.39)	
Unfiltered Water Consumption (cups per day)						
≤ 2	64	62.75	32	65.31	32	60.38
> 2	38	37.25	17	34.69	21	39.62
Unfiltered Water Consumption						
Mean (SD)	2.0 (2.36)		2.02 (2.60)		1.98 (2.15)	
Dose***						
Atrazine (Syngenta)						
≤ 0.20	12	41.38	n/a	n/a	12	41.38
> 0.20	17	58.62	n/a	n/a	17	58.62
Chlorotriazine (Syngenta)						
≤ 0.43	10	34.48	n/a	n/a	10	34.48
> 0.43	19	65.52	n/a	n/a	19	65.52
Atrazine (CDC)						
≤ 0.36	11	28.95	8	38.1	3	17.65
> 0.36	27	71.05	13	61.9	14	82.35
Chlorotriazine (CDC)						
≤ 2.50	8	21.05	5	23.81	3	17.65
> 2.50	30	78.95	16	76.19	14	82.35

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation. SD, standard deviation.

Table 3.15. Menstrual cycle characteristics of the study population; total population and stratified by state

	TOTAL		*Vermont*		*Illinois*	
Characteristics	***N=102***	***Percent***	***N=49***	***Percent***	***N=53***	***Percent***
Age at Menarche (years) (Questionnaire)						
12-13	60	58.82	32	60.38	28	57.14
<12 or >13	42	41.18	21	39.62	21	42.86
Dysmenorrhea (Questionnaire)						
Never or Sometimes	39	38.24	16	32.65	23	43.4
Often or Always	63	61.76	33	67.35	30	56.6
Menstrual Cycle Regular Length (Questionnaire)						
Yes	76	76.77	43	89.58	33	64.71
No	23	23.23	5	10.42	18	35.29
Predict onset of period within 4 days (Questionnaire)						
Yes	73	72.28	39	81.25	34	64.15
No	28	27.72	9	18.75	19	35.85
Cycle Length (days) (Questionnaire)						
< 24	6	5.88	4	8.16	2	3.77
25-30	74	72.55	36	73.47	38	71.7
31-35	12	11.76	6	12.24	6	11.32
36-42	4	3.92	1	2.04	3	5.66
> 43	1	0.98	0	0	1	1.89
Too irregular to say	5	4.9	2	4.08	3	5.66
Cycle Length (days) (Diary)						
< 24	5	7.46	3	8.11	2	6.67
25-30	41	61.19	24	64.86	17	56.67
31-35	12	17.91	5	13.51	7	23.33
36-42	2	2.99	2	5.41	0	0.00
> 43	7	10.45	3	8.11	4	13.33
Cycle Length (days) (Diary)						
Mean (SD)	31.30 (10.38)		30.46 (6.78)		32.33 (13.64)	
Did you ever go more than 6 weeks without a menstrual period? (Questionnaire)						
Yes	13	12.75	2	4.08	11	20.75
No	89	87.25	47	95.92	42	79.25
Did you ever bleed or spot between menstrual periods? (Questionnaire)						
Yes	13	12.75	5	10.20	8	15.10
No	89	87.25	44	89.80	45	84.90

SD, standard deviation

Table 3.16. Hormone concentrations and phase lengths; total population and stratified by state.

	TOTAL		Vermont	Illinois
Hormone	**N**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Preovulatory LH (mIU/mg Cr)	33	17.39 (11.00)	18.45 (10.68)	15.77 (11.71)
Mid-luteal Phase $E_1$3G (ng/mg Cr)	35	28.26 (13.08)	31.34 (13.29)	24.15 (12.01)
Follicular Phase Pd3G (μg/mg Cr)	35	0.91 (0.78)	0.98 (0.99)	0.82 (0.36)
Mid-luteal Phase Pd3G (μg/mg Cr)	35	10.42 (5.29)	10.93 (6.06)	9.73 (4.15)
LH Surge Peak (mIU/mg Cr)	33	52.58 (19.97)	55.02 (19.56)	49.27 (20.78)
Follicular Phase Length (days)	35	15.94 (5.12)	16.0 (5.48)	15.87 (4.79)
Luteal Phase Length (days)	35	12.91 (1.56)	12.95 (1.43)	12.87 (1.77)

SD, standard deviation; Cr, creatinine

Table 3.17. Logistic regression analyses between between markers of atrazine exposure and menstrual cycle length irregularity** as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	**cases, controls**	**OR**	**95% CI**	**P-value**	**cases, controls**	**OR**	**95% CI**	**P-value**
State of Residence								
Vermont	5,43	1.00	-	-	4,43	1.0	-	-
Illinois	18,33	4.69	1.58, 13.95	0.005	18,33	4.45†	1.32, 15.01	0.02
Years in current home (Vermont only)§								
≤ 4 (Vermont)	2,15	1.0	-	-		*	-	-
> 4	3,28	0.80	0.12, 5.35	0.82				
Years in current home (Illinois and Vermont)§								
Vermont	5,43	1.0	-	-	4,42	1.0	-	-
≤ 4 (*Illinois*)	6,18	2.87	0.78, 10.60	0.11	5,17	2.05‡	0.44, 9.57	0.36
> 4	12,15	6.88	2.08, 22.78	0.002	11,13	8.55‡	2.16, 33.96	0.002
Years in current home (*Illinois only*)§								
≤ 4 (Illinois)	6,18	1.0	-	-	5,17	1.0	-	-
> 4	12,15	2.40	0.73, 7.94	0.15	11,13	4.16¶	0.92, 18.94	0.06
Consumption of Unfiltered Water (Vermont only)§								
≤ 2 (Vermont)	3,29	1.0	-	-		*	-	-
> 2	2,14	1.38	0.21, 9.23	0.74				
Consumption of Unfiltered Water (Illinois and Vermont)§§								
Vermont	5,43	1.0	-	-	4,43	1.0	-	-
≤ 2 (Illinois)	10,21	4.10	1.24, 13.51	0.02	10,21	5.14#	1.31, 20.17	0.02
> 2	8,12	5.73	1.58, 20.77	0.01	8,12	6.56#	1.38, 31.10	0.02
Consumption of Unfiltered Water (Illinois only)§§								
≤ 2 (Illinois)	10,21	1.0	-	-	10,21	1.0	-	-
> 2	8,12	1.4	0.44, 4.51	0.57	8,12	1.12††	0.31, 3.99	0.86

OR, odds ratio; CI, confidence interval.

**Assessed by the question, "Generally speaking, are your periods regular or irregular? That is, is the length of time between the first day of one period and the first day of the other about the same each cycle?."

§ Levels determined by median of Illinois controls.

* Frequencies < 5, adjusted ORs not calculated

† Adjusted for age (continuous) and BMI (<25/≥25).

‡ Adjusted for age (continuous), BMI (<25/≥25), and income (<$60,000/≥ $60,000).

¶ Adjusted for parity (yes/no), and income (<$60,000/≥ $60,000).

Adjusted for age (continuous), BMI (<25/≥25) and education (college graduate/non college graduate).

†† Adjusted for age (continuous).

§§ Assessed by the question "During a typical day at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?" Levels determined by median of Illinois controls.

Table 3.18. Logistic regression analyses between between markers of atrazine exposure and going more than 6 weeks without a menstrual period** as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	**cases, controls**	**OR**	**95% CI**	**P-value**	**cases, controls**	**OR**	**95% CI**	**P-value**
State of Residence								
Vermont	2,47	1.0	-	-		*	-	-
Illinois	11,42	6.16	1.29, 29.38	0.02				
Length of time in current home (Vermont only)§								
≤ 4 (Vermont)	0,18	1.0†	-	-		*	-	-
> 4	2,29	-	-	-				
Years in current home (Illinois and Vermont)§								
Vermont	2,47	1.0	-	-		*	-	-
≤ 4 (Illinois)	7,17	9.68	1.83, 51.22	0.01				
> 4	4,25	3.76	0.64, 21.97	0.14				
Years in current home (Illinois only)§								
≤ 4 (Illinois)	7,17	1.0	-	-		*	-	-
> 4	4,25	0.39	0.10, 1.54	0.18				
Consumption of Unfiltered Water (Vermont only)§§								
≤ 2 (Vermont)	0,32	1.0†	-	-		*	-	-
> 2	2,15	-	-	-				
Consumption of Unfiltered Water (Illinois and Vermont)§§								
Vermont	2,47	1.0	-	-		*	-	-
≤ 2 (Illinois)	8,24	7.83	1.54, 39.81	0.01				
> 2	3,18	3.92	0.60, 25.41	0.15				
Consumption of Unfiltered Water (Illinois only)§§								
≤ 2 (Illinois)	8,24	1.0	-	-		*	-	-
> 2	3,18	0.50	0.12, 2.16	0.35				

OR, odds ratio; CI, confidence interval.

**Assessed by the question, "During the last 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills."

* Frequencies < 5, adjusted ORs not calculated

§ Levels determined by median of Illinois controls.

§§ Assessed by the question "During a typical day at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?" Levels determined by median of Illinois controls.

Table 3.19. Logistic regression analyses between between markers of atrazine exposure and inter-menstrual bleeding as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	cases, controls	OR	95% CI	P-value	cases, controls	OR	95% CI	P-value
State of Residence								
Vermont	5,44				5,43			
Illinois	8,45	1.56	0.48, 5.15	0.46	8,38	2.07†	0.61, 7.06	0.24
Length of time in current home (Vermont only)§								
≤ 4 (Vermont)	3,15	1.0	-	-		*	-	-
> 4	2,29	0.35	0.05, 2.29	0.27				
Years in current home (Illinois and Vermont)§								
Vermont	5,44	1.0	-	-		*	-	-
≤ 4 (Illinois)	3,21	1.26	0.27, 5.76	0.77				
> 4	5,24	1.83	0.48, 6.97	0.37				
Years in current home (Illinois only)§								
≤ 4 (Illinois)	3,21	1.0	-	-		*	-	-
> 4	5,24	1.46	0.31, 6.85	0.63				
Consumption of Unfiltered Water (Vermont only)§§								
≤ 2 (Vermont)	3,29	1.0	-	-		*	-	-
> 2	2,15	1.29	0.19, 8.57	0.79				
Consumption of Unfiltered Water (Illinois and Vermont)§§								
Vermont	5,44	1.0	-	-		*	-	-
≤ 2 (Illinois)	4,28	1.26	0.31, 5.09	0.75				
> 2	4,17	2.07	0.50, 8.64	0.32				
Consumption of Unfiltered Water (Illinois only)§§								
≤ 2 (Illinois)	4,28	1.0	-	-		*	-	-
> 2	4,17	1.65	0.36, 7.47	0.52				

OR, odds ratio; CI, confidence interval.

**Assessed by the question, "During the last 12 months did you ever bleed or spot between menstrual periods (Do not count times when you were pregnant, breast feeding or using birth control pills/medication)?"

* Frequencies < 5, adjusted ORs not calculated

† Adjusted for vegetable consumption (≤1 serving/day vs. >1 serving/day) and income (<$60,000 vs. ≥ $60,000)

§ Levels determined by median of Illinois controls.

§§ Assessed by the question "During a typical day at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?" Levels determined by median of Illinois controls.

Table 3.20. Logistic regression analyses between between markers of atrazine exposure and menstrual cycle length** as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	**cases, controls**	**OR**	**95% CI**	**P-value**	**cases, controls**	**OR**	**95% CI**	**P-value**
State of Residence								
Vermont	7,40	1.0	-	-	7,40	1.0	-	-
Illinois	10,40	1.43	0.50, 4.13	0.51	10,40	1.17†	0.36, 3.79	0.79
Length of time in current home (*Vermont only*)§								
≤ 4 (Vermont)	2,16	1.0	-	-		*	-	-
> 4	5,24	1.67	0.29, 9.66	0.57				
Years in current home (Illinois and Vermont)§								
Vermont	9,40	1.0	-	-		*	-	-
≤ 4 (Illinois)	5,19	1.17	0.35, 3.97	0.80				
> 4	8,21	1.69	0.57, 5.03	0.34				
Years in current home (Illinois only)§								
≤ 4 (Illinois)	5,19	1.0	-	-		*	-	-
> 4	8,21	1.45	0.40, 5.20	0.57				
Consumption of Unfiltered Water (Vermont only)§§								
≤ 2 (Vermont)	4,28	1.0	-	-		*	-	-
> 2	3,12	1.75	0.34, 9.05	0.50				
Consumption of Unfiltered Water (Illinois and Vermont)§§								
Vermont	9,40	1.0	-	-		*	-	-
≤ 2 (Illinois)	8,24	1.48	0.50, 4.36	0.48				
> 2	5,16	1.39	0.40, 4.79	0.60				
Consumption of Unfiltered Water (Illinois only)§§								
≤ 2 (Illinois)	8,24	1.0	-	-		*	-	-
> 2	5,16	0.94	0.26, 3.39	0.92				

OR, odds ratio; CI, confidence interval.

**Assessed by the question, "Many women have their periods about once a month. Some women have their periods more often and others less often. How often are your menstrual periods? In other words, how many days are there from the first day of one menstrual period to the first day of the next period?" The categorical response choices ≤ 24 days and 25-30 days were considered not long while 31-35, 36-42 and 43 days or more were considered long.

* Frequencies < 5, adjusted ORs not calculated

† Adjusted for age (continuous) and smoking (currently/not currently)

§ Levels determined by median of Illinois controls.

§§ Assessed by the question "During a typical day at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?" Levels determined by median of Illinois controls.

Table 3.21. Logistic regression analyses between markers of atrazine exposure and menstrual cycle cramps** as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	**cases, controls**	**OR**	**95% CI**	**P-value**	**cases, controls**	**OR**	**95% CI**	**P-value**
State of Residence								
Vermont	33,16	1.0	-	-				
Illinois	30,23	0.63	0.28, 1.42	0.27		*	-	-
Length of time in current home (Vermont only)§								
≤ 4 (Vermont)	9,9	1.0	-	-		*	-	-
> 4	24,7	3.43	0.98, 11.97	0.05				
Years in current home (Illinois and Vermont)§								
Vermont	33,16	1.0	-	-	32,16	1.0	-	-
≤ 4 (Illinois)	11,13	0.41	0.15, 1.12	0.06	10,11	0.46†	0.15, 1.38	0.17
>4	19,10	0.92	0.35, 2.43	0.87	8,7	1.05†	0.37, 2.99	0.93
Years in current home (Illinois only)§								
≤ 4 (Illinois)	11,13	1.0	-	-	11,12	1.0	-	-
>4	19,10	2.55	0.82, 7.89	0.11	19,10	2.29‡	0.69, 7.61	0.18
Consumption of Unfiltered Water (Vermont only)§§								
≤ 2 (Vermont)	18,14	1.0	-	-		*	-	-
> 2	15,2	5.83	1.14, 29.84	0.03				
Consumption of Unfiltered Water (Illinois and Vermont)§§								
Vermont	33,16	1.0	-	-	32,16	1.0	-	-
≤ 2 (Illinois)	20,12	0.81	0.32, 2.05	0.65	19,9	1.09¶	0.38, 3.07	0.88
> 2	10,11	0.44	0.16, 1.25	0.12	8,10	0.25¶	0.07, 0.89	0.03
Consumption of Unfiltered Water (Illinois only)§§								
≤ 2 (Illinois)	20,12	1.0	-	-	19,10	1.0	-	-
> 2	10,11	0.55	0.18, 1.67	0.29	8,10	0.34#	0.09, 1.23	0.10

OR, odds ratio; CI, confidence interval.

**Assessed by the question, "Approximately, how often do you have cramps or backache with your menstrual periods?" The categorical response choices 'never' and 'sometimes' were considered normal while 'often' and 'always' were considered not normal.

* Frequencies < 5 or confounding not found to be present, adjusted ORs not calculated

§ Levels determined by median of Illinois controls.

† Adjusted for income (<$60,000 vs. ≥ $60,000), caffeine consumption (<300 vs. ≥300 mg/day) and vegetable consumption

‡ Adjusted for caffeine consumption (<300 vs. ≥300 mg/day) and vegetable consumption (≤1 serving/day vs. >1 serving/day).

¶ Adjusted for income (<$60,000 vs. ≥ $60,000), caffeine consumption (<300 vs. ≥300 mg/day), vegetable consumption (≤1 serving/day vs. >1 serving/day) and age (continuous).

Adjusted for income (<$60,000 vs. ≥ $60,000) and caffeine consumption (<300 vs. ≥300 mg/day).

§§ Assessed by the question "During a typical day at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?" Levels determined by median of Illinois controls.

Table 3.22. Differences in mean menstrual cycle length (days) as reported by prospective diary for atrazine (and markers of atrazine).

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	37	30.46	6.78	
Illinois	30	32.33	13.64	0.50
Years in current home (Illinois & Vermont)				
Vermont	37	30.46	6.78	
≤ 4 (Illinois)	12	28.50	2.81	0.16
> 4	18	34.89	17.17	0.30
Years in current home (Illinois only)				
≤ 4	12	28.50	2.81	
> 4	18	34.89	17.17	0.14
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	37	30.46	6.78	
≤ 2	15	31.80	18.03	0.70
> 2	14	33.00	8.00	0.26
Unfiltered Water Consumption (Illinois only)#				
≤ 2	15	31.80	18.03	
> 2	14	33.00	8.00	0.82
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	20	30.00	5.35	
> 0.36	16	31.06	7.40	0.62
Atrazine (without chlorine)				
≤ 0.36	20	30.00	5.35	
> 0.36	16	31.06	7.40	0.62
Chlorotriazine (with chlorine)				
≤ 2.50	33	30.76	6.47	
> 2.50	3	27.33	1.53	0.37
Chlorotriazine (without chlorine)				
≤ 2.50	26	31.23	7.16	
> 2.50	10	28.50	2.12	0.09

Table 3.22. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (Desethylatrazine mercapturate)				
≤ 0.36	12	30.50	4.62	
> 0.36	24	30.46	7.04	0.99
Syngenta Monitoring§§				
Atrazine				
≤ 0.20	16	30.81	7.47	
> 0.20	14	34.07	18.56	0.55
Chlorotriazine				
≤ 0.43	14	29.86	7.07	
> 0.43	16	34.50	17.48	0.34
Dose* **				
Atrazine (Syngenta)				
≤ 0.20	12	32.67	20.19	
> 0.20	17	32.18	7.54	0.94
Chlorotriazine (Syngenta)				
≤ 0.43	10	33.80	22.12	
> 0.43	19	31.63	7.29	0.77
Atrazine (CDC)				
≤ 0.36	11	30.91	7.11	
> 0.36	24	30.25	6.14	0.78
Chlorotriazine (CDC)				
≤ 2.50	11	30.91	7.11	
> 2.50	24	30.25	6.14	0.78

SD, standard deviation

*p-value comparing the menstrual cycle means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/√2 and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.23. Linear regression analyses of atrazine (and markers of atrazine) and menstrual cycle length* (days) as reported by prospective diary.

	Unadjusted				Adjusted			
Exposure	**n**	**β coefficient§**	**95% CI§**	**P-value**	**n**	**β coefficient§**	**95% CI§**	**P-value**
State of Residence								
Vermont	37				37			
Illinois	30	-0.18	-2.30, 1.95	0.87	30	0.70†	-1.39, 2.78	0.51
Years in current home (Illinois & Vermont)								
Vermont	37				37			
≤ 4 (Illinois)	12	0.63	-2.25, 3.52	0.66	12	1.41†	-1.36, 4.17	0.31
> 4	18	-0.72	-3.21, 1.78	0.57	18	0.21†	-2.22, 2.64	0.86
Years in current home (Illinois only)								
≤ 4	12				10			
> 4	18	-1.35	-4.93, 2.24	0.45	15	-0.42‡	-4.12, 3.28	0.82
Unfiltered Water Consumption (Illinois & Vermont)#								
Vermont	37				37			
≤ 2	16	1.12	-1.43, 3.67	0.38	16	1.85†	-0.61, 4.30	0.14
> 2	14	-1.66	-4.33, 1.02	0.22	14	-0.68†	-3.28, 1.93	0.61
Unfiltered Water Consumption (Illinois only)#								
≤ 2	16							
> 2	14	-2.77	-6.16, 0.61	0.10		NC		
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	20				20			
> 0.36	16	-0.32	-2.48, 1.84	0.76	16	0.18†	-1.80, 2.16	0.85
Atrazine (without chlorine)								
≤ 0.36	20				20			
> 0.36	16	-0.32	-2.48, 1.84	0.76	16	0.18†	-1.80, 2.16	0.85
Chlorotriazine (with chlorine)								
≤ 2.50	33							
> 2.50	3	1.95	-1.88, 5.78	0.31		NC		
Chlorotriazine (without chlorine)								
≤ 2.50	26							
> 2.50	10	1.15	-1.21, 3.52	0.33		NC		

Table 3.23. Continued

Exposure	Unadjusted n	Unadjusted β coefficient§	Unadjusted 95% CI§	Unadjusted P-value	Adjusted n	Adjusted β coefficient§	Adjusted 95% CI§	Adjusted P-value
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12				10			
> 0.36	24	0.65	-1.62, 2.91	0.57	23	-0.35‡	-3.08, 2.39	0.80
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	16				16			
> 0.20	14	0.24	-3.32, 3.79	0.89	14	-0.25¶	-3.50, 3.00	0.88
Chlorotriazine								
≤ 0.43	14				14			
> 0.43	16	-0.84	-4.38, 2.70	0.63	11	1.55‡	-2.26, 5.36	0.41
Dose* **								
Atrazine (Syngenta)								
≤ 0.20	12							
> 0.20	17	-2.65	-6.23, 0.93	0.14		NC		
Chlorotriazine (Syngenta)								
≤ 0.43	10							
> 0.43	19	-2.28	-6.03, 1.48	0.22		NC		
Atrazine (CDC)								
≤ 0.36	11				11			
> 0.36	24	0.12	-2.26, 2.50	0.92	21	-0.29‡	-2.81, 2.24	0.82
Chlorotriazine (CDC)								
≤ 2.50	11				11			
> 2.50	24	0.12	-2.26, 2.50	0.92	21	-0.29‡	-2.81, 2.24	0.82

§ x 10,000

CI, confidence interval; NC, no confounding present.

* Inverse squared transformed.

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

† Adjusted for age (continuous).

‡ Adjusted for income (<$60,000/≥ $60,000).

¶ Adjusted for caffeine consumption (<300 vs. ≥300 mg/day).

** Average of the two residential tap water samples dichotomized at limit of detection/√2 and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.24. Differences in mean urinary preovulatory luteinizing hormone levels for atrazine (and markers of atrazine).

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	20	18.45	10.68	
Illinois	13	15.77	11.71	0.50
Years in current home (Illinois & Vermont)				
Vermont	20	18.45	10.68	
≤ 4 (Illinois)	6	18.59	15.83	0.98
> 4	7	13.35	7.12	0.25
Years in current home (Illinois only)				
≤ 4	6	18.59	15.83	
> 4	7	13.35	7.12	0.45
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	20	18.45	10.68	
≤ 2	5	18.53	15.26	0.99
> 2	8	14.04	9.66	0.32
Unfiltered Water Consumption (Illinois only)#				
≤ 2	5	18.53	15.26	
> 2	8	14.04	9.66	0.53
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	20	18.45	10.68	
> 0.36	13	15.77	11.71	0.50
Atrazine (without chlorine)				
≤ 0.36	20	18.45	10.68	
> 0.36	13	15.77	11.71	0.50
Chlorotriazine (with chlorine)				
≤ 2.50	30	17.28	9.98	
> 2.50	3	18.54	22.50	0.93
Chlorotriazine (without chlorine)				
≤ 2.50	24	15.31	7.21	
> 2.50	9	22.96	16.93	0.22

Table 3.24. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (Desethylatrazine mercapturate)				
≤ 0.36	12	15.57	6.81	
> 0.36	21	18.43	12.84	0.41
Syngenta Monitoring§§				
Atrazine				
≤ 0.20	11	14.07	8.47	
> 0.20	2	25.08	26.90	0.67
Chlorotriazine				
≤ 0.43	8	17.38	14.36	
> 0.43	5	13.18	6.08	0.55
Dose* **				
Atrazine (Syngenta)				
≤ 0.20	5	18.34	15.45	
> 0.20	8	14.16	9.54	0.55
Chlorotriazine (Syngenta)				
≤ 0.43	3	23.79	18.50	
> 0.43	10	13.36	8.88	0.19
Atrazine (CDC)				
≤ 0.36	11	19.16	10.69	
> 0.36	22	16.51	11.29	0.52
Chlorotriazine (CDC)				
≤ 2.50	11	19.16	10.69	
> 2.50	22	16.51	11.29	0.52

SD, standard deviation

*p-value comparing preovulatory luteinizing hormone means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.25. Linear regression analyses of atrazine (and markers of atrazine)and urinary preovulatory luteinizing hormone* levels.

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
State of Residence								
Vermont	20							
Illinois	13	-0.26	-0.68, 0.17	0.23		NC		
Years in current home (Illinois & Vermont)								
Vermont	20							
≤ 4 (Illinois)	6	-0.17	-0.74, 0.39	0.54				
> 4	7	-0.33	-0.86, 0.21	0.22		NC		
Years in current home (Illinois only)								
≤ 4	6							
> 4	7	-0.16	-1.03, 0.72	0.70		NC		
Unfiltered Water Consumption (Illinois & Vermont)†								
Vermont	20							
≤ 2	5	-0.11	-0.72, 0.49	0.70				
> 2	8	-0.34	-0.85, 0.16	0.18		NC		
Unfiltered Water Consumption (Illinois only)†								
≤ 2	5							
> 2	8	-0.23	-1.12, 0.66	0.58		NC		
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	19							
> 0.36	14	-0.26	-0.68, 0.17	0.23		NC		
Atrazine (without chlorine)								
≤ 0.36	19							
> 0.36	14	-0.26	-0.68, 0.17	0.23		NC		
Chlorotriazine (with chlorine)								
≤ 2.50	30							
> 2.50	3	-0.28	-1.02, 0.45	0.44		NC		
Chlorotriazine (without chlorine)								
≤ 2.50	23							
> 2.50	10	0.27	-0.20, 0.74	0.25		NC		

Table 3.25. Continued

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12							
> 0.36	21	0.06	-0.38, 0.51	0.77		NC		
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	12							
> 0.20	2	0.31	-0.89, 1.51	0.58		NC		
Chlorotriazine								
≤ 0.43	9							
> 0.43	5	-0.06	-0.97, 0.84	0.88		NC		
Dose* **								
Atrazine (Syngenta)								
≤ 0.20	5							
> 0.20	8	-0.18	-1.07, 0.72	0.67		NC		
Chlorotriazine (Syngenta)								
≤ 0.43	3							
> 0.43	10	-0.53	-1.51, 0.45	0.26		NC		
Atrazine (CDC)								
≤ 0.36	10							
> 0.36	22	-0.21	-0.66, 0.23	0.34		NC		
Chlorotriazine (CDC)								
≤ 2.50	10							
> 2.50	22	-0.21	-0.66, 0.23	0.34		NC		

SE, standard error; CI, confidence interval; NC, no confounding present.

* Log transformed.

† Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.26. Differences in mean urinary mid-luteal estrone 3-glucuronide levels for atrazine (and markers of atrazine).

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	20	31.34	13.29	
Illinois	15	24.15	12.01	0.11
Years in current home (Illinois & Vermont)				
Vermont	20	31.34	13.29	
≤ 4 (Illinois)	7	26.59	13.43	0.42
> 4	8	22.02	11.08	0.09
Years in current home (Illinois only)				
≤ 4	7	26.59	13.43	
> 4	8	22.02	11.08	0.48
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	20	31.34	13.29	
≤ 2	6	24.54	16.71	0.31
> 2	9	23.90	8.82	0.14
Unfiltered Water Consumption (Illinois only)#				
≤ 2	6	24.54	16.71	
> 2	9	23.90	8.82	0.92
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	20	31.34	13.29	
> 0.36	15	24.15	12.01	0.11
Atrazine (without chlorine)				
≤ 0.36	20	31.34	13.29	
> 0.36	15	24.15	12.01	0.11
Chlorotriazine (with chlorine)				
≤ 2.50	32	28.15	12.47	
> 2.50	3	29.49	22.24	0.87
Chlorotriazine (without chlorine)				
≤ 2.50	25	26.18	10.39	
> 2.50	10	33.46	17.79	0.25

Table 3.26. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (Desethylatrazine mercapturate)				
≤ 0.36	12	26.90	13.25	
> 0.36	23	28.97	13.23	0.66
Syngenta Monitoring§§				
Atrazine				
≤ 0.20	13	23.00	10.44	
> 0.20	2	31.66	24.13	0.36
Chlorotriazine				
≤ 0.43	10	22.90	12.94	
> 0.43	5	26.66	10.81	0.59
Dose* **				
Atrazine (Syngenta)				
≤ 0.20	6	29.45	13.98	
> 0.20	9	20.62	9.76	0.17
Chlorotriazine (Syngenta)				
≤ 0.43	4	32.99	13.24	
> 0.43	11	20.94	10.33	0.09
Atrazine (CDC)				
≤ 0.36	12	35.67	12.14	
> 0.36	23	24.39	12.05	0.01
Chlorotriazine (CDC)				
≤ 2.50	12	35.67	12.14	
> 2.50	23	24.39	12.05	0.01

SD, standard deviation

*p-value comparing mid-luteal estrone 3-glucuronide means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.27. Linear regression analyses of atrazine (and markers of atrazine) and urinary mid-luteal estrone 3-glucuronide* levels.

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
State of Residence								
Vermont	20							
Illinois	15	-0.32	-0.68, 0.04	0.08		NC		
Years in current home (Illinois & Vermont)								
Vermont	20							
≤ 4 (Illinois)	7	-0.20	-0.66, 0.27	0.40				
> 4	8	-0.43	-0.87, 0.02	0.06		NC		
Years in current home (Illinois only)								
≤ 4	7							
> 4	8	-0.23	-0.93, 0.46	0.48		NC		
Unfiltered Water Consumption (Illinois & Vermont)†								
Vermont	20							
≤ 2	6	-0.41	-0.91, 0.09	0.10				
> 2	9	-0.26	-0.69, 0.17	0.23		NC		
Unfiltered Water Consumption (Illinois only)†								
≤ 2	6							
> 2	9	0.16	-0.56, 0.87	0.65		NC		
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	20							
> 0.36	15	-0.32	-0.68, 0.04	0.08		NC		
Atrazine (without chlorine)								
≤ 0.36	20							
> 0.36	15	-0.32	-0.68, 0.04	0.08		NC		
Chlorotriazine (with chlorine)								
≤ 2.50	32							
> 2.50	3	-0.22	-0.88, 0.45	0.51		NC		
Chlorotriazine (without chlorine)								
≤ 2.50	25							
> 2.50	10	0.20	-0.21, 0.61	0.33		NC		

Table 3.27. Continued

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12							
> 0.36	23	0.07	-0.32, 0.47	0.71		NC		
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	13							
> 0.20	2	0.28	-0.74, 1.31	0.56		NC		
Chlorotriazine								
≤ 0.43	10							
> 0.43	5	0.22	-0.52, 0.96	0.52		NC		
Dose* **								
Atrazine (Syngenta)								
≤ 0.20	5							
> 0.20	9	-0.37	-1.06, 0.31	0.26		NC		
Chlorotriazine (Syngenta)								
≤ 0.43	3							
> 0.43	11	-0.53	-1.27, 0.20	0.14		NC		
Atrazine (CDC)								
≤ 0.36	11							
> 0.36	23	-0.46	-0.82, -0.10	0.01		NC		
Chlorotriazine (CDC)								
≤ 2.50	11							
> 2.50	23	-0.46	-0.82, -0.10	0.01		NC		

SE, standard error; CI, confidence interval; NC, no confounding present.

* Log transformed.

† Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.28. Differences in mean urinary mid-luteal pregnanediol 3-glucuronide levels for atrazine (and markers of atrazine).

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	20	10.93	6.06	
Illinois	15	9.73	4.15	0.51
Years in current home (Illinois & Vermont)				
Vermont	20	10.93	6.06	
≤ 4 (Illinois)	7	11.55	4.70	0.81
> 4	8	8.13	3.06	0.23
Years in current home (Illinois only)				
≤ 4	7	11.55	4.70	
> 4	8	8.13	3.06	0.11
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	20	10.93	6.06	
≤ 2	6	11.24	3.21	0.91
> 2	9	8.72	4.57	0.34
Unfiltered Water Consumption (Illinois only)#				
≤ 2	6	11.24	3.21	
> 2	9	8.72	4.57	0.27
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	20	10.93	6.06	
> 0.36	15	9.73	4.15	0.51
Atrazine (without chlorine)				
≤ 0.36	20	10.93	6.06	
> 0.36	15	9.73	4.15	0.51
Chlorotriazine (with chlorine)				
≤ 2.50	32	10.39	5.42	
> 2.50	3	10.63	4.66	0.94
Chlorotriazine (without chlorine)				
≤ 2.50	25	9.50	5.28	
> 2.50	10	12.70	4.83	0.11

Table 3.28. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (Desethylatrazine mercapturate)				
≤ 0.36	12	9.84	3.80	
> 0.36	23	10.72	5.98	0.65
Syngenta Monitoring§§				
Atrazine				
≤ 0.20	13	9.87	3.92	
> 0.20	2	8.78	7.41	0.74
Chlorotriazine				
≤ 0.43	10	9.11	4.03	
> 0.43	5	10.97	4.59	0.44
Dose* **				
Atrazine (Syngenta)				
≤ 0.20	6	12.44	1.38	
> 0.20	9	7.92	4.45	0.02
Chlorotriazine (Syngenta)				
≤ 0.43	4	12.08	1.55	
> 0.43	11	8.87	4.52	0.20
Atrazine (CDC)				
≤ 0.36	12	11.94	5.08	
> 0.36	23	9.62	5.33	0.22
Chlorotriazine (CDC)				
≤ 2.50	12	11.94	5.08	
> 2.50	23	9.62	5.33	0.22

SD, standard deviation

*p-value comparing mid-luteal pregnanediol 3-glucuronide means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/√2 and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.29. Linear regression analyses of atrazine (and markers of atrazine) and urinary mid-luteal pregnanediol 3-glucuronide* levels.

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
State of Residence								
Vermont	20							
Illinois	15	-0.06	-0.46, 0.33	0.75		NC		
Years in current home (Illinois & Vermont)								
Vermont	20							
≤ 4 (Illinois)	7	0.11	-0.40, 0.62	0.67				
> 4	8	-0.21	-0.69, 0.27	0.38		NC		
Years in current home (Illinois only)								
≤ 4	7							
> 4	8	-0.32	-0.87, 0.23	0.24		NC		
Unfiltered Water Consumption (Illinois & Vermont)†								
Vermont	20							
≤ 2	6	0.14	-0.40, 0.68	0.60				
> 2	9	-0.20	-0.66, 0.27	0.39		NC		
Unfiltered Water Consumption (Illinois only)†								
≤ 2	6							
> 2	9	-0.34	-0.89, 0.22	0.22		NC		
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	20							
> 0.36	15	-0.06	-0.46, 0.33	0.75		NC		
Atrazine (without chlorine)								
≤ 0.36	20							
> 0.36	15	-0.06	-0.46, 0.33	0.75		NC		
Chlorotriazine (with chlorine)								
≤ 2.50	32							
> 2.50	3	0.08	-0.62, 0.78	0.81		NC		
Chlorotriazine (without chlorine)								
≤ 2.50	25							
> 2.50	10	0.34	-0.07, 0.76	0.10		NC		

Table 3.29. Continued

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12							
> 0.36	23	0.03	-0.38, 0.45	0.88		NC		
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	12							
> 0.20	1	-0.25	-1.09, 0.59	0.53		NC		
Chlorotriazine								
≤ 0.43	10							
> 0.43	5	0.23	-0.37, 0.83	0.43		NC		
Dose* **								
Atrazine (Syngenta)								
≤ 0.20	5							
> 0.20	9	-0.57	-1.06, -0.09	0.02		NC		
Chlorotriazine (Syngenta)								
≤ 0.43	3							
> 0.43	11	-0.43	-1.03, 0.18	0.15		NC		
Atrazine (CDC)								
≤ 0.36	11							
> 0.36	23	-0.31	-0.71, 0.08	0.12		NC		
Chlorotriazine (CDC)								
≤ 2.50	11							
> 2.50	23	-0.31	-0.71, 0.08	0.12		NC		

SE, standard error; CI, confidence interval; NC, no confounding present.

* Log transformed.

† Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/√2 and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.30. Differences in mean urinary luteinizing hormone peak levels for atrazine (and markers of atrazine).

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	19	55.02	19.56	
Illinois	14	49.27	20.78	0.42
Years in current home (Illinois & Vermont)				
Vermont	19	55.02	19.56	
≤ 4 (Illinois)	6	45.22	21.85	0.31
> 4	8	52.31	20.89	0.75
Years in current home (Illinois only)				
≤ 4	6	45.22	21.85	
> 4	8	52.31	20.89	0.55
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	19	55.02	19.56	
≤ 2	6	49.70	27.31	0.60
> 2	8	48.94	16.40	0.45
Unfiltered Water Consumption (Illinois only)#				
≤ 2	6	49.70	27.31	
> 2	8	48.94	16.40	0.95
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	19	55.02	19.56	
> 0.36	14	49.27	20.78	0.42
Atrazine (without chlorine)				
≤ 0.36	19	55.02	19.56	
> 0.36	14	49.27	20.78	0.42
Chlorotriazine (with chlorine)				
≤ 2.50	30	53.88	18.52	
> 2.50	3	39.63	33.70	0.25
Chlorotriazine (without chlorine)				
≤ 2.50	23	46.37	17.41	
> 2.50	10	66.86	18.77	0.005

Table 3.30. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (Desethylatrazine mercapturate)				
≤ 0.36	12	50.87	20.66	
> 0.36	21	53.56	20.02	0.72
Syngenta Monitoring§§				
Atrazine	12	44.53	18.41	
≤ 0.20	21	77.69	0.77	0.03
> 0.20				
Chlorotriazine				
≤ 0.43	9	49.82	25.25	
> 0.43	5	48.27	11.23	0.90
Dose* **				
Atrazine (Syngenta)	6	51.11	25.39	
≤ 0.20	8	47.89	18.34	0.79
> 0.20				
Chlorotriazine (Syngenta)				
≤ 0.43	4	63.34	21.74	
> 0.43	10	43.64	18.52	0.11
Atrazine (CDC)				
≤ 0.36	11	55.99	17.16	
> 0.36	22	50.87	21.41	0.50
Chlorotriazine (CDC)				
≤ 2.50	11	55.99	17.16	
> 2.50	22	50.87	21.41	0.50

SD, standard deviation

*p-value comparing luteinizing hormone peak means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.31. Linear regression analyses of atrazine (and markers of atrazine) and urinary luteinizing hormone* peak levels.

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
State of Residence								
Vermont	19							
Illinois	14	-0.15	-0.44, 0.15	0.32		NC		
Years in current home (Illinois & Vermont)								
Vermont	19							
≤ 4 (Illinois)	6	-0.23	-0.63, 0.17	0.25				
> 4	8	-0.09	-0.44, 0.27	0.63		NC		
Years in current home (Illinois only)								
≤ 4	6							
> 4	8	0.14	-0.44, 0.72	0.60		NC		
Unfiltered Water Consumption (Illinois & Vermont)†								
Vermont	19							
≤ 2	6	-0.20	-0.60, 0.20	0.32				
> 2	8	-0.11	-0.47, 0.25	0.54		NC		
Unfiltered Water Consumption (Illinois only)†								
≤ 2	6							
> 2	8	0.09	-0.50, 0.67	0.74		NC		
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	20							
> 0.36	13	-0.15	-0.44, 0.15	0.32		NC		
Atrazine (without chlorine)								
≤ 0.36	20							
> 0.36	13	-0.15	-0.44, 0.15	0.32		NC		
Chlorotriazine (with chlorine)								
≤ 2.50	30							
> 2.50	3	-0.48	-0.97, 0.00	0.05		NC		
Chlorotriazine (without chlorine)								
≤ 2.50	24							
> 2.50	9	0.39	0.10, 0.68	0.01		NC		

Table 3.31. Continued

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12							
> 0.36	21	0.05	-0.26, 0.36	0.75		NC		
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	11							
> 0.20	2	0.64	-0.08, 1.37	0.08		NC		
Chlorotriazine								
≤ 0.43	8							
> 0.43	5	0.09	-0.52, 0.69	0.76		NC		
Dose* **								
Atrazine (Syngenta)								
≤ 0.20	4							
> 0.20	8	-0.03	-0.62, 0.55	0.91		NC		
Chlorotriazine (Syngenta)								
≤ 0.43	2							
> 0.43	10	-0.40	-1.00, 0.19	0.16		NC		
Atrazine (CDC)								
≤ 0.36	10							
> 0.36	22	-0.14	-0.45, 0.17	0.38		NC		
Chlorotriazine (CDC)								
≤ 2.50	10							
> 2.50	22	-0.14	-0.45, 0.17	0.38		NC		

SE, standard error; CI, confidence interval; NC, no confounding present.

* Log transformed.

† Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/√2 and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.32. Differences in mean urinary follicular pregnanediol 3-glucuronide levels for atrazine (and markers of atrazine).

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	20	0.98	0.99	
Illinois	15	0.82	0.36	0.51
Years in current home (Illinois & Vermont)				
Vermont	20	0.98	0.99	
≤ 4 (Illinois)	7	1.01	0.41	0.92
> 4	8	0.66	0.22	0.18
Years in current home (Illinois only)				
≤ 4	7	1.01	0.41	
> 4	8	0.66	0.22	0.06
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	20	0.98	0.99	
≤ 2	6	0.68	0.17	0.21
> 2	9	0.91	0.43	0.80
Unfiltered Water Consumption (Illinois only)#				
≤ 2	6	0.68	0.17	
> 2	9	0.91	0.43	0.23
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	20	0.98	0.99	
> 0.36	15	0.82	0.36	0.51
Atrazine (without chlorine)				
≤ 0.36	20	0.98	0.99	
> 0.36	15	0.82	0.36	0.51
Chlorotriazine (with chlorine)				
≤ 2.50	32	0.93	0.81	
> 2.50	3	0.71	0.13	0.65
Chlorotriazine (without chlorine)				
≤ 2.50	25	0.95	0.90	
> 2.50	10	0.81	0.30	0.50

Table 3.32. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (Desethylatrazine mercapturate)				
≤ 0.36	12	0.71	0.26	
> 0.36	23	1.02	0.93	0.14
Syngenta Monitoring§§				
Atrazine				
≤ 0.20	13	0.86	0.36	
> 0.20	2	0.57	0.31	0.31
Chlorotriazine				
≤ 0.43	10	0.82	0.35	
> 0.43	5	0.83	0.41	0.95
Dose* **				
Atrazine (Syngenta)				
≤ 0.20	6	0.72	0.17	
> 0.20	9	0.89	0.44	0.32
Chlorotriazine (Syngenta)				
≤ 0.43	4	0.67	0.19	
> 0.43	11	0.88	0.39	0.33
Atrazine (CDC)				
≤ 0.36	12	1.14	1.25	
> 0.36	23	0.79	0.33	0.36
Chlorotriazine (CDC)				
≤ 2.50	12	1.14	1.25	
> 2.50	23	0.79	0.33	0.36

SD, standard deviation

*p-value comparing follicular pregnanediol 3-glucuronide means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.33. Linear regression analyses of atrazine (and markers of atrazine) and urinary follicular pregnanediol 3-glucuronide* levels.

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
State of Residence								
Vermont	20							
Illinois	15	-0.05	-0.43, 0.34	0.80		NC		
Years in current home (Illinois & Vermont)								
Vermont	20							
≤ 4 (Illinois)	7	0.16	-0.33, 0.65	0.50				
> 4	8	-0.23	-0.70, 0.23	0.32		NC		
Years in current home (Illinois only)								
≤ 4	7							
> 4	8	-0.39	-0.86, 0.07	0.09		NC		
Unfiltered Water Consumption (Illinois & Vermont)†								
Vermont	20							
≤ 2	6	-0.18	-0.70, 0.35	0.50				
> 2	9	0.04	-0.42, 0.49	0.87		NC		
Unfiltered Water Consumption (Illinois only)†								
≤ 2	6							
> 2	9	0.21	-0.30, 0.73	0.38		NC		
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	20							
> 0.36	15	-0.05	-0.43, 0.34	0.80		NC		
Atrazine (without chlorine)								
≤ 0.36	20							
> 0.36	15	-0.05	-0.43, 0.34	0.80		NC		
Chlorotriazine (with chlorine)								
≤ 2.50	32							
> 2.50	3	-0.10	-0.77, 0.58	0.78		NC		
Chlorotriazine (without chlorine)								
≤ 2.50	25							
> 2.50	10	-0.02	-0.44, 0.40	0.93		NC		

Table 3.33. Continued

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12							
> 0.36	23	0.28	-0.11, 0.67	0.15		NC		
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	13							
> 0.20	2	-0.40	-1.12, 0.32	0.25		NC		
Chlorotriazine								
≤ 0.43	10							
> 0.43	5	0.01	-0.54, 0.56	0.97		NC		
Dose***								
Atrazine (Syngenta)								
≤ 0.20	5							
> 0.20	9	0.12	-0.40, 0.64	0.63		NC		
Chlorotriazine (Syngenta)								
≤ 0.43	3							
> 0.43	11	0.21	-0.36, 0.78	0.44		NC		
Atrazine (CDC)								
≤ 0.36	11							
> 0.36	23	-0.23	-0.62, 0.17	0.25		NC		
Chlorotriazine (CDC)								
≤ 2.50	11							
> 2.50	23	-0.23	-0.62, 0.17	0.25		NC		

SE, standard error; CI, confidence interval; NC, no confounding present.

* Log transformed.

† Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.34. Differences in mean follicular phase length for atrazine exposures and markers of exposure.

Exposure	n	Mean	SD	p-value*
State of Residence				
Vermont	20	16.00	5.48	
Illinois	15	15.87	4.79	0.94
Years in current home (Illinois & Vermont)				
Vermont	20	16.00	5.48	
≤ 4 (Illinois)	7	14.00	3.42	0.38
> 4	8	17.50	5.42	0.52
Years in current home (Illinois only)				
≤ 4	7	14.00	3.42	
> 4	8	17.50	5.42	0.17
Unfiltered Water Consumption (Illinois & Vermont)#				
Vermont	20	16.00	5.48	
≤ 2	6	14.00	3.69	0.41
> 2	9	17.10	5.23	0.61
Unfiltered Water Consumption (Illinois only)#				
≤ 2	6	14.00	3.69	
> 2	9	17.10	5.23	0.23
Residential tap water**				
Atrazine (with chlorine)				
≤ 0.36	20	16.00	5.48	
> 0.36	15	15.87	4.79	0.94
Atrazine (without chlorine)				
≤ 0.36	20	16.00	5.48	
> 0.36	15	15.87	4.79	0.94
Chlorotriazine (with chlorine)				
≤ 2.50	32	16.16	5.30	
> 2.50	3	13.67	1.53	0.43
Chlorotriazine (without chlorine)				
≤ 2.50	25	16.40	5.79	
> 2.50	10	14.80	2.78	0.28

Table 3.34. Continued

Exposure	n	Mean	SD	p-value*
Urinary biomarker (*Desethylatrazine mercapturate*)				
≤ 0.36	12	16.17	4.65	
> 0.36	23	15.83	5.45	0.86
Syngenta Monitoring§§				
Atrazine				
≤ 0.20	13	16.08	5.04	
> 0.20	2	14.50	3.54	0.68
Chlorotriazine				
≤ 0.43	10	14.30	2.91	
> 0.43	5	19.00	6.56	0.19
Dose* **				
Atrazine (Syngenta)				
≤ 0.20	6	13.83	3.66	
> 0.20	9	17.22	5.17	0.19
Chlorotriazine (Syngenta)				
≤ 0.43	4	14.00	4.69	
> 0.43	11	16.55	4.87	0.38
Atrazine (CDC)				
≤ 0.36	12	16.92	7.08	
> 0.36	23	15.43	3.84	0.51
Chlorotriazine (CDC)				
≤ 2.50	12	16.92	7.08	
> 2.50	23	15.43	3.84	0.51

SD, standard deviation

*p-value comparing follicular phase length means, atrazine exposure versus lowest atrazine exposure

Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.35. Linear regression analyses of atrazine (and markers of atrazine) and follicular phase length.

	Unadjusted				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
State of Residence								
Vermont	20				20			
Illinois	15	0.000	-0.01, 0.01	0.99	15	0.002†	-0.01, 0.01	0.78
Years in current home (Illinois & Vermont)								
Vermont	20				20			
≤ 4 (Illinois)	7	0.008	-0.01, 0.02	0.28	7	0.011†	0.00, 0.02	0.13
> 4	8	-0.007	-0.02, 0.01	0.32	8	-0.006†	-0.02, 0.01	0.35
Years in current home (Illinois only)								
≤ 4	7				7			
> 4	8	-0.015	-0.03, 0.00	0.10	8	-0.017†	-0.03 0.00	0.05
Unfiltered Water Consumption (Illinois & Vermont)§								
Vermont	20				20			
≤ 2	6	0.008	-0.01, 0.02	0.27	6	0.01†	-0.01, 0.02	0.19
> 2	9	-0.005	-0.02, 0.01	0.41	9	-0.004†	-0.02, 0.01	0.53
Unfiltered Water Consumption (Illinois only)§								
≤ 2	6				6			
> 2	9	-0.014	-0.03, 0.00	0.13	9	-0.014†	-0.03, 0.00	0.12
Residential tap water**								
Atrazine (with chlorine)								
≤ 0.36	20				20			
> 0.36	15	0.000	-0.01, 0.01	0.99	15	0.002†	-0.01, 0.01	0.78
Atrazine (without chlorine)								
≤ 0.36	20				20			
> 0.36	15	0.000	-0.01, 0.01	0.99	15	0.002†	-0.01, 0.01	0.78
Chlorotriazine (with chlorine)								
≤ 2.50	32				32			
> 2.50	3	0.007	-0.01, 0.03	0.49	3	0.008†	-0.01, 0.03	0.42
Chlorotriazine (without chlorine)								
≤ 2.50	25				25			
> 2.50	10	0.003	-0.01, 0.02	0.58	10	0.001†	-0.01, 0.01	0.85

Table 3.35. Continued

	Crude				Adjusted			
Exposure	*n*	*β*	*95% CI*	*P-value*	*n*	*β*	*95% CI*	*P-value*
Urinary biomarker (Desethylatrazine mercapturate)								
≤ 0.36	12				12			
> 0.36	23	0.004	-0.01, 0.02	0.53	23	0.004†	-0.01, 0.02	0.50
Syngenta Monitoring§§								
Atrazine								
≤ 0.20	13				13			
> 0.20	2	0.004	-0.03, 0.03	0.76	2	-0.000†	-0.03, 0.03	0.98
Chlorotriazine								
≤ 0.43	10				10			
> 0.43	5	-0.016	-0.03, 0.00	0.09	5	-0.019†	-0.04, 0.00	0.04
Dose***								
Atrazine (Syngenta)								
≤ 0.20	5				5			
> 0.20	9	-0.021	-0.04, 0.00	0.03	9	-0.018†	-0.04, 0.00	0.05
Chlorotriazine (Syngenta)								
≤ 0.43	3				3			
> 0.43	11	-0.023	-0.04, 0.00	0.03	11	-0.02†	-0.04, 0.00	0.08
Atrazine (CDC)								
≤ 0.36	11				11			
> 0.36	23	-0.001	-0.01, 0.01	0.81	23	-0.000†	-0.01, 0.01	0.99
Chlorotriazine (CDC)								
≤ 2.50	11				11			
> 2.50	23	-0.001	-0.01, 0.01	0.81	23	-0.00†	-0.01, 0.01	0.99

SE, standard error; CI, confidence interval; NC, no confounding present.

* Inverse transformed.

§ Assessed by the question "During a typical day while at home, how many (unfiltered) glasses of plain water (and powdered or concentrated water) do you drink at home?"

† Adjusted for parity.

** Average of the two residential tap water samples dichotomized at limit of detection/$\sqrt{2}$ and analyzed by CDC.

§§ A temporally weighted average of the two Syngenta monitoring results closest to the date of participation imputed for each woman. Dichotomized using a median split.

*** Calculated by multiplying the volume of unfiltered water ingested per day by the concentration of atrazine (and chlorotriazine) in drinking water. Both the imputed Syngenta values and the residential tap water averages analyzed by CDC were used for the concentrations in the dose exposure metric calculation.

Table 3.36. Linear regression analyses between follicular phase length, luteal phase length and & peak pregnanediol 3-glucuronide and menstrual cycle length (days) as reported by prospective menstrual cycle diary.

	Unadjusted					Adjusted				
Exposure	**n**	**β coefficient**	**SE**	**95% CI**	**P-value**	**n**	**β coefficient**	**SE**	**95% CI**	**P-value**
Follicular Phase Length	35	0.93	0.05	0.82, 1.04	<.0001		NC			
Luteal Phase Length	35	0.05	0.56	-1.08, 1.19	0.92		NC			
Peak Pregnanediol 3-Glucuronide	32	-0.09	0.15	-0.39, 0.21	0.54		NC			

SE, standard error; CI, confidence interval; NC, no confounding

Table 3.37. Logistic regression analyses between phase follicular phase length, luteal phase length & mid luteal pregnanediol 3-glucuronide and menstrual cycle length regularity* as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	**cases, controls**	**OR**	**95% CI**	**P-value**	**cases, controls**	**OR**	**95% CI**	**P-value**
Follicular Phase Length	3, 30	1.10	0.93, 1.31	0.28		**		
Luteal Phase Length3, 3	0	0.90	0.43, 1.88	0.78		**		
Peak Pregnanediol 3-Glucuronide	1, 29	0.99	0.69, 1.42	0.97		**		

* Assessed by the question, "Generally speaking, are your periods regular or irregular? That is, is the length of time between the first day of one period and the first day of the other about the same each cycle?"
OR, odds ratio; CI, confidence interval.

** Frequencies < 5, adjusted ORs not calculated.

Table 3.38. Logistic regression analyses between phase follicular phase length, luteal phase length & mid luteal pregnanediol 3-glucuronide and going more than 6 weeks without a menstrual period* as reported by retrospective questionnaire.

	Crude				Adjusted			
Exposure	**cases, controls**	**OR**	**95% CI**	**P-value**	**cases, controls**	**OR**	**95% CI**	**P-value**
Follicular Phase Length	2, 33	0.33	0.11, 1.00	0.05		**		
Luteal Phase Length	2, 3	3	3.77	0.91, 15.59	0.07		**	
Peak Pregnanediol 3-Glucuronide	2, 30	1.05	0.82, 1.34	0.71		**		

* Assessed by the question, "During the last 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills."

OR, odds ratio; CI, confidence interval.

** Frequencies < 5, adjusted ORs not calculated.

Table 3.39. Demographic characteristics and regularity for women with concordant and discordant responses for normal cycle lengths defined as every 25-35 days.

	Total (*n*= 67)	*Discordant* (*n*=11)	*Concordant* (*n*=56)	*OR (95% CI)* (concordant vs. discordant)
	mean (standard deviation)			
Age (years)	33.4 (5.6)	34.5 (5.2)	33.2 (5.7)	-1.31 (-5.00-2.38)
Body Mass Index	26.2 (5.7)	24.5 (5.0)	26.5 (5.8)	1.97 (-1.77-5.72)
	number (percent)			
Education				
< College	36 (53.7)	8 (11.9)	28 (41.8)	2.67 (0.64-11.10)
≥ College†	31 (46.3)	3 (4.5)	28 (41.8)	
Income				
< $60,000	37 (60.7)	6 (9.8)	31 (50.8)	0.74 (0.20-2.75)
≥ $60,000†	24 (39.3)	5 (8.2)	19 (31.2)	
Alcohol				
≥ 2 drinks/week	15 (22.4)	4 (6.0)	11 (16.4)	2.34 (0.58-9.42)
< 2 drinks/week†	52 (77.6)	7 (10.5)	45 (67.2)	
Smoking				
Ever	27 (40.3)	3 (4.5)	24 (35.8)	0.50 (0.12-2.09)
Never†	40 (59.7)	8 (11.9)	32 (47.8)	
Physical activity				
≥ 6 hours	7 (10.5)	2 (3.0)	5 (7.5)	2.27 (0.38-13.53)
< 6 hours†	60 (89.6)	9 (13.4)	51 (76.1)	
Regularity				
<25 or >35	16 (23.9)	8 (11.9)	8 (11.9)	16.0 (3.49-73.41)
25-35†	51 (76.1)	3 (4.5)	48 (71.6)	

† comparison group

Table 3.40. Number of women reporting a normal menstrual cycle length on the retrospective questionnaire versus the prospective menstrual diary and the corresponding percent agreement, kappa, and prevalence index when normal cycle length is defined as 25-30 days and 25-35 days.

		25-30 days			**25-35 days**		
		Prospective Diary			**Prospective Diary**		
		Normal Menstrual Cycle			Normal Menstrual Cycle		
Retrospective Questionnaire							
Normal Menstrual Cycle		No	Yes	Total	No	Yes	Total
	No	12	7	19	6	3	9
	Yes	14	34	48	8	50	58
	Total	26	41	67	14	53	67
Percent Agreement				68.7%			83.6%
Kappa				0.31			0.43
Prevalence Index				-0.33			-0.66

Figure 3.1. Study Participants

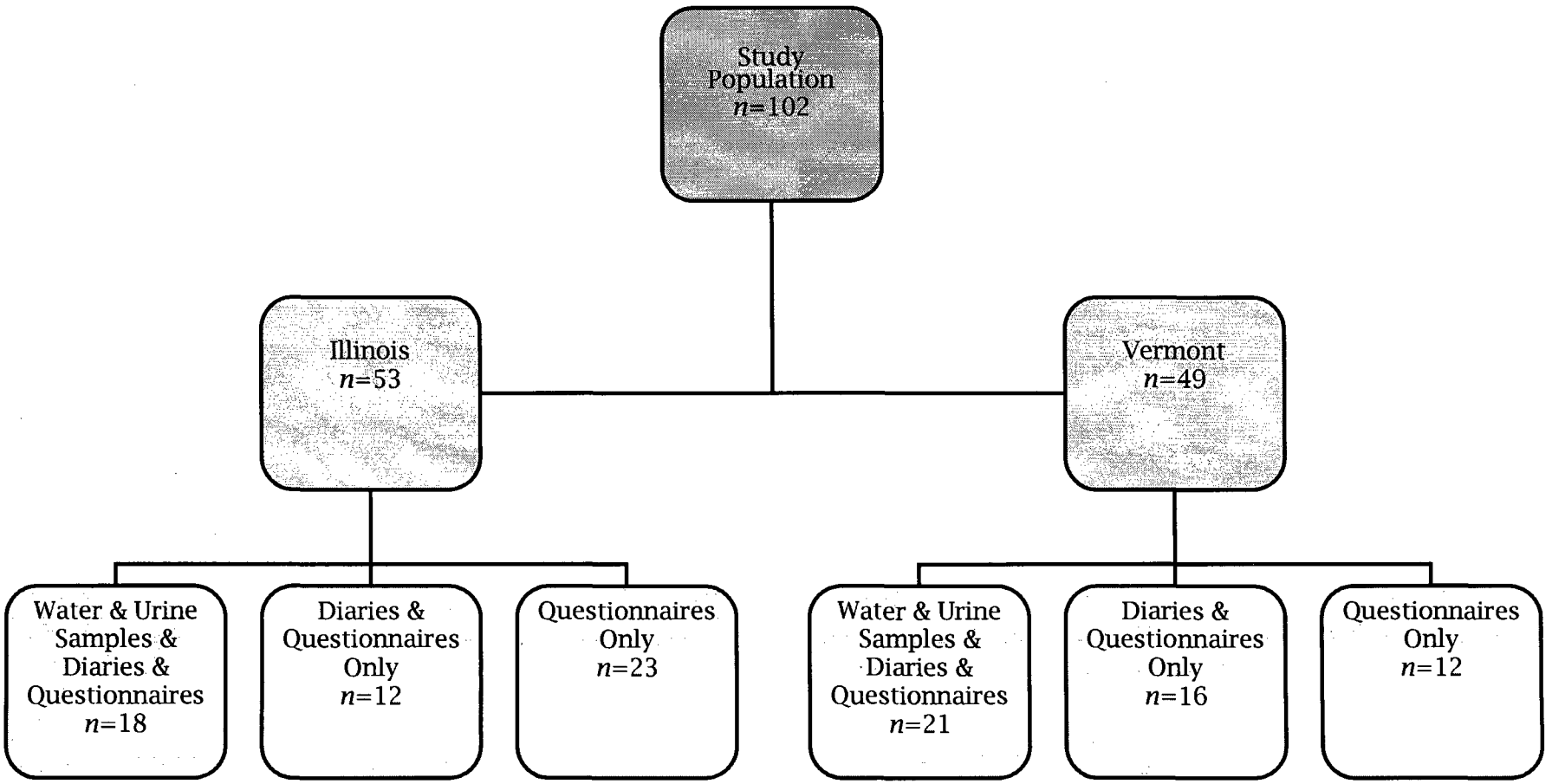
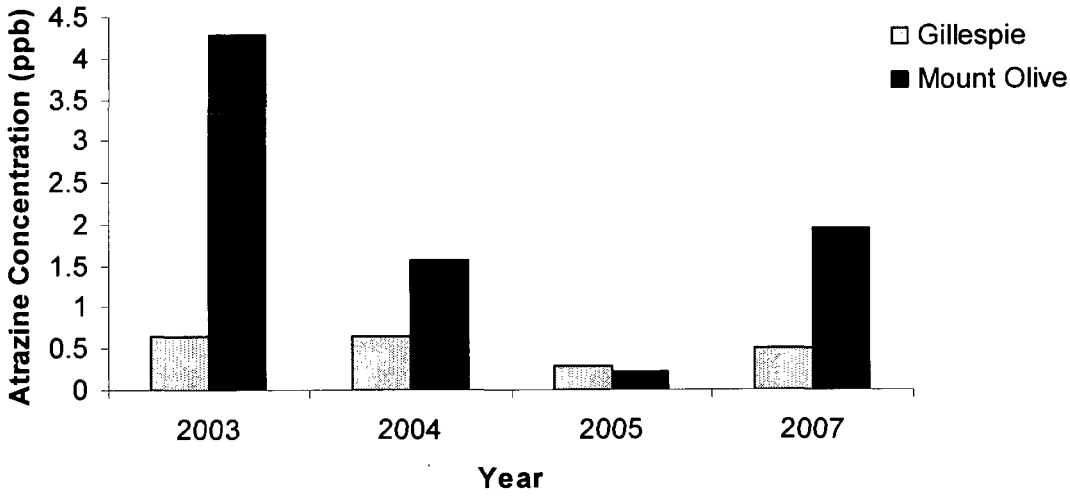
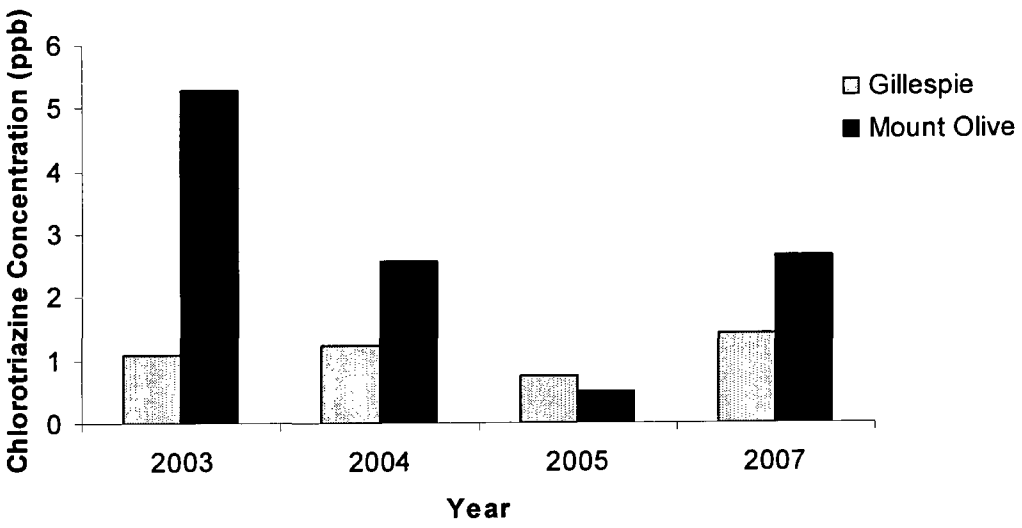


Figure 3.2. Syngenta Municipal Plant Atrazine Monitoring by Year.



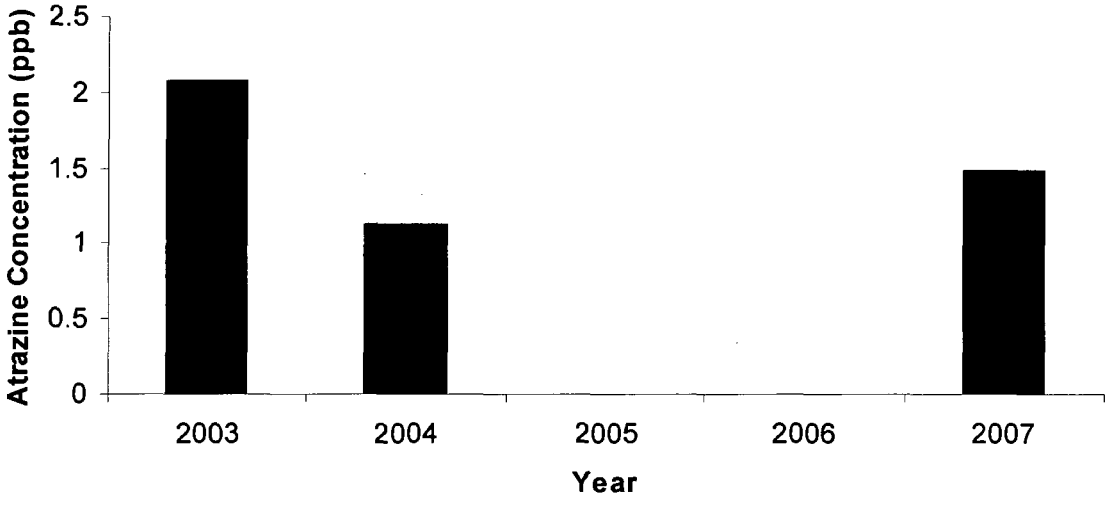
Note: Data are yearly atrazine averages. No data were available for 2006.

Figure 3.3. Syngenta Municipal Plant Chlorotriazine Monitoring by Year.



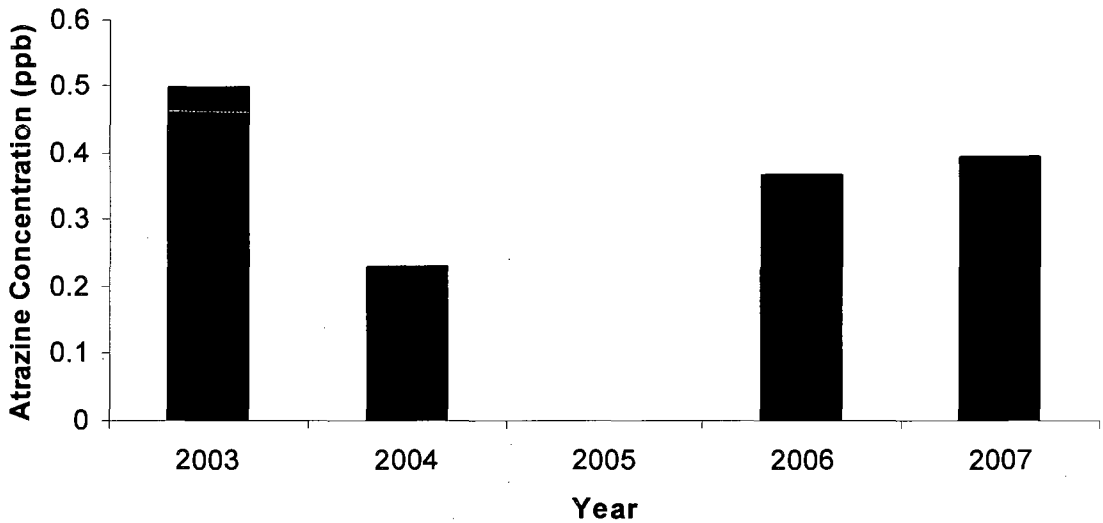
Note: Data are yearly atrazine averages. No data were available for 2006.

Figure 3.4. Illinois EPA community water system atrazine monitoring for Mount Olive, Illinois by Year.



Note: Data are yearly atrazine averages. Non-detections were calculated as zero.

Figure 3.5. Illinois EPA community water system atrazine monitoring for Gillespie, Illinois by Year.



Note: Data are yearly atrazine averages. Non-detections were calculated as zero.

5. Discussion

This study was the first to examine potential associations between exposure to atrazine in drinking water and menstrual cycle characteristics in women. The communities of Mount Olive and Gillespie, Illinois were selected as exposed site locations after reviewing data from the 2003 Atrazine Monitoring Program (Syngenta Crop Protection, Inc.). Monitoring was required in these communities because past atrazine municipal plant drinking water levels exceeded the EPA MCL of 3.0 ppb. These two communities had among the highest atrazine drinking water concentrations out of 28 municipal water systems required to be monitored in Illinois in 2003. Mount Olive had raw water concentrations of 18.8 ppb and 20.6 ppb for atrazine and chlorotriazine, respectively. Gillespie had raw water concentrations of 5.11 ppb and 7.23 ppb for atrazine and chlortriazine, respectively. Mount Olive treated (finished) water concentrations were more than three times the EPA standard with levels as high as 9.8 ppb and 11.9 ppb for atrazine and chlorotriazine, respectively. The concentrations of atrazine and chlorotriazine for Gillespie's treated water were 1.18 ppb and 1.75 ppb.

Because of the widespread atrazine use and presence in municipal drinking water across the mid-West, the corn-belt of the country, it was not possible to select unexposed study sites that were in close proximity to the exposed sites. Instead, communities in Vermont were selected since Vermont is outside of the Corn Belt, yet, still considered an agricultural state. Prior to data collection, demographic data from the 2000 census suggested Waterbury and Fair Haven, Vermont were similar to Gillespie and Mount

Olive, Illinois with regards to age, race and income. After data collection, potentially confounding characteristics were again compared between Vermont and Illinois. For the most part, characteristics were similar between the two states, although Vermont women were older and slightly more educated.

Data obtained from municipal monitoring by both by Syngenta Crop Protection, Inc. and the Illinois EPA showed that drinking water atrazine concentrations were uniquely low in 2005, the year of data collection for this study. Historically, annual average exposure levels below 1.0 ppb chlorotriazine were not typical for these areas of Illinois. Higher atrazine drinking water concentrations were observed in both preceding and subsequent years. Municipal plant atrazine monitoring revealed water samples of 4.4 ppb in 2003 and 1.8 in 2004 (Illinois EPA quarterly monitoring water data) and levels of 4.3 ppb in 2003 and 1.6 ppb in 2004 (Syngenta Crop Protection, Inc.) in Mount Olive, Illinois. However, in 2005, according to the Illinois EPA water data, municipal plant samples never exceeded the limit of detection. Municipal monitoring conducted by Syngenta Crop Protection, Inc., revealed an annual average for atrazine of 0.51 ppb in 2005. Atrazine concentrations in municipal water were again elevated in 2007 in Mount Olive, according to both Illinois EPA quarterly monitoring water data and Syngenta Crop Protection, Inc. water data. Gillespie, Illinois also had no measurable levels in 2005 but atrazine was present in both preceding and subsequent years, as indicated by Illinois EPA quarterly monitoring water data.

According to the tap water measurements analyzed by CDC for this study, fewer than 45% of samples analyzed in 2005 had atrazine levels above the limit of detection (0.5 ppb), with no atrazine result greater than 1.83 ppb. Additionally, as indicated by the results of this study, the average 2005 chlorotriazine tap water level was 2.90 ppb, which is just below the current EPA MCL of 3.0 ppb.

Results of atrazine urine analyses concur with the low 2005 atrazine drinking water concentrations. According to Barr et al. (93), only the presence of atrazine or atrazine mercapturate in urine definitively indicates an individual was previously exposed to the parent compound atrazine (and not an atrazine breakdown product such as desisopropylatrazine or desethylatrazine). In this study, only two out of 39 women (5%) had measurable urinary atrazine levels, suggesting they were exposed to the parent compound atrazine in the recent past since the half-life of atrazine in water is approximately 6 months (80). The predominant metabolite detected in this study (and the only metabolite detected with measurable levels in more than 15.0% of urine samples) was DEAM. Although the presence of DEAM does not indicate exposure to the parent compound atrazine, it does indicate exposure to one of the dealkylated atrazine breakdown products which, in addition to atrazine, are also considered biologically active (93). Therefore, results of urine analyses indicate women in this study were being exposed to atrazine breakdown products. This suggests that exposure occurred some time earlier when levels may have been higher.

Exposure to atrazine was assessed in multiple ways including indirect assessment (also referred to as surrogate, or group exposure assessment), direct assessment (also referred to as individual assessment), and by the use of biological markers of exposure (202). State of residence was used as a surrogate marker of exposure for some analyses which resulted in unknown sensitivity and specificity of the exposure assignment. Exposure uncertainty was present when using state of residence since it was assumed all women in the exposure group had the same exposure. Exposure misclassification could have also occurred when using state of residence if, for example, women in Illinois consumed only bottled water or filtered tap water potentially leaving them unexposed to municipal drinking water atrazine concentrations. Years in current home, although an individual-level measurement, was not a measure of actual drinking water atrazine concentration. The potential for exposure misclassification also exists for this marker since years in current home did not measure the amount of water consumed or the amount of atrazine in the water. Since actual amount of water being ingested was taken into account with unfiltered water consumption as an exposure variable, the exposure assessment was improved. However, the potential for exposure misclassification remained. Unfiltered water consumption was based on an average of consumption not current consumption given that women did not maintain diaries of actual current beverage intake and the consumption of unfiltered water variable relied on retrospective questionnaire data. In addition, the concentration of atrazine in the water was not taken into account at this stage when the unfiltered water consumption variable was used in the analyses. For each of the above described markers of exposure since they are dichotomous, an y misclassification most likely would have been indiscriminate and independent of disease

status (i.e., non-differential or random between cases and controls) and, therefore, attenuated any association (i.e., biased the results toward the null), although this assumes confounders were not misclassified which is not often the case.

The exposure assessment using residential water samples measured atrazine (and chlorotriazine) concentrations in the tap water at each woman's home. The exposure assessment using Syngenta Crop Protection, Inc. water data relied on the atrazine (and chlorotriazine) concentrations reported at the municipal plant (municipal plant concentrations were imputed for each woman based on her date of participation). So, although both of these assessments use individual-level data neither of these exposure metrics measured or estimated the amount of water consumed. Therefore, misclassification of exposure may exist since 'dose' was not taken into account.

Exposure estimated using 'dose' presumably represented an improvement over all other exposure metrics used in this study since it was based on atrazine concentration measured in residential tap water (CDC water data) as well as an estimate of the amount of unfiltered water consumed (questionnaire data). Exposure misclassification biases are reduced by developing exposure indices that integrate individual-level data and consumption patterns resulting in a more accurate and precise representation of exposure.

Nonetheless, exposure misclassification was likely to be present for all metrics used in this study. However, it was also likely to have been non-differential (both the cases and the controls are misclassified in the same manner) and, since there were only two

exposure categories, any bias would have attenuated the association or biased the results toward the null (again, however, this assumes other variables such as confounders were not misclassified which seldom happens).

Biological markers of exposure are measures of internal dose capable of reducing misclassification bias and thereby, improving estimates of effect (203). They are objective and individualized. However, since the half-life of atrazine in humans is approximately eight hours and it is metabolized quickly, atrazine does not circulate in the body for a prolonged amount of time. Atrazine also does not accumulate in the body. Therefore, a limitation of using an atrazine biomarker for exposure assessment is that it only represents exposures occurring in the immediate past (204). For exposures that are chronic and, therefore, eliminated in urine at a steady rate, a biomarker would characterize exposure over a greater time period (204). This is not the case with atrazine since levels fluctuate throughout the year as well as by year. In addition to representing one point in time, biomarkers for atrazine drinking water exposure also take into consideration the amount of water ingested the day before which may or may not be indicative of an individual's usual drinking water consumption pattern. The validity of internal dose is further complicated by individual variations in chemical metabolism due to such factors as age, genetics, and organ function (205). Rate of metabolism could also depend on an individual's exposure to other chemicals (205). Biomarkers of exposure were used in this study in an effort to improve exposure assessment. First morning voids were collected but since the half-life of atrazine is generally eight hours and complete elimination typically occurs in 24 hours, complete daily urine voids may improve

exposure assessment for atrazine (or any compound rapidly metabolized). Additionally, CDC was unable to determine the hydroxylated metabolites and two fo the glutathione metabolites in urine samples. Therefore, although the exposure assessment in this study was improved over past studies since an increased number of atrazine metabolites were examined in urine, it would be possible to further improve the exposure assessment by analyzing for other metabolites (i.e., diaminotriazine mercapturate and deisopropyl atrazine mercapturate and the hydroxylated metabolites: hydroxyatrazine, atrazine mercapturate, and hydroxydesethylatrazine).

Previous studies have reported human exposures to atrazine at higher concentrations. Ikonen et al. (206) reported occupational exposure to atrazine in railway men responsible for removing weeds from railway lines with urinary metabolite concentrations as high as 23,726 ppb. Custom applicators in Ohio who applied herbicides to corn and soybean fields while enclosed in cabs, had urinary triazine mean concentrations of 3.9 ppb (207). Swan et al. (208) reported 6% of men in Minnesota where pesticide use was low had atrazine mercapturate levels above the limit of detection (0.1 μg/g Cr) while almost 40% had detectable levels in Missouri where pesticide use was higher. None of the women in this study had measurable atrazine mercapturate urinary levels. Perry et al. (209) reported 37% of applicators in Wisconsin had detectable levels of desethylatrazine with mean levels of 14.2 ppb. In this study, the mean level of desethylatrazine was 0.84 ppb. Overall, the levels of exposure to atrazine found in the current study were much lower than the levels reported in previous occupational and environmental studies.

Exposures of atrazine used in toxicological studies can be compared to doses for women in this study on a mg/kg body weight basis. Variable doses ranging from 2 mg/kg to 300 mg/kg were administered to animals in studies examining atrazine's association with hormones (61, 84, 135, 136, 140). For this study, based on a 62 kg female consuming 2 liters of water per day and an average atrazine concentration in tap water of 0.62 ppb, women were 'dosed' with 0.00002 mg/kg of atrazine per day. Therefore, the animal doses at which hormonal effects were seen in past studies were 100,000 to 15,000,000 times higher than the average 'dose' of atrazine received by women in this study.

Recruitment of participants for this study was challenging. A large number of women (82%) were ineligible due to the extensive list of exclusion criteria developed in an effort to control for confounding. It was particularly difficult to find women between the ages of 18 and 40 who were not taking oral contraceptives. These constraints resulted in a small sample size.

Overall, 53% of women refused to participate. Of the 102 women that participated in the study, 35 (34%) participated by only answering the questionnaire and 28 (27%) participated by maintaining diaries and answering questionnaires. Thirty-nine (38%) participated by providing urine and water samples for exposure and hormone analyses (i.e., the participation level where expectations were greatest). The expectations of the participating women were high and minimal compensation was provided; however, only two women were lost to follow-up during the urine collection. Although the possibility of selection bias cannot be excluded, there is no reason to believe women with menstrual

cycle abnormalities who were exposed to atrazine were more likely to participate in the study (resulting in a bias away from the null) or less likely to participate in the study (resulting in a bias towards the null). Many of the women in Illinois did not know atrazine was present in their water or that particular water filtration systems (i.e., charcoal) they may be using removed it. Additionally, the rates of infertility, number of pregnancies, number of live births and certain menstrual cycle characteristics were similar between the two states, indicating women from Illinois (where exposure was greater) with certain 'conditions' were not participating at an increased rate. Furthermore, in an effort to reduce the likelihood of self-selection bias, women were recruited for a reproductive health study and were not informed that the purpose of the study was to specifically investigate menstrual cycle characteristics or drinking water exposures.

Prior to this study, Farr et al. (25) conducted the only epidemiologic study that assessed the association between menstrual cycle function and atrazine exposure, although exposure was assessed by lifetime use (i.e., mixing or applying) of atrazine. Farr et al. (25) found that women who had used either atrazine or lindane (two pesticides the authors classified as probable hormonally active) in their lifetime reported longer menstrual cycles. In addition to long cycles, Farr et al. (25) reported missed periods and intermenstrual bleeding associated with using lindane, atrazine, mancozeb or maneb.

Findings of this study were in agreement with Farr et al. (25). A consistent association was observed between menstrual cycle length irregularity and atrazine exposure, as estimated in several ways. Using state of residence as an exposure marker, women living

in Illinois were more likely to report irregular menstrual cycle lengths. In addition, the length of time the women lived in their homes in Illinois was significantly associated with irregular cycle lengths, with a dose response effect observed. A dose response association was also observed between the amount of unfiltered water consumed and menstrual length irregularity.

Consistent with the findings of Farr et al. (25), this study also found that going more than six weeks without a menstrual period was associated with atrazine exposure (both residence in Illinois and residing for more than four years in current home) although small numbers made it difficult to draw strong conclusions. Previous epidemiologic studies have also reported associations between endocrine disruptors and increased cycle length (23, 24, 29). Exposure to organic solvents was associated with increased cycle length (> 35 days) in a cross-sectional study of 1,408 petrochemical workers in China (29). Additionally, Cooper et al. (23) reported an increase in menstrual cycle length among women exposed to PCBs. Eskenazi et al. (24) reported a lengthened menstrual cycle was associated with exposure to dioxin (TCDD, tetrachlorodibenzodioxin) among women who were premenarcheal at the time of exposure. The authors speculated TCDD was acting as an endocrine disruptor possibly by lowering the gonadotropin levels and increasing the length of the follicular or secretory phase (24).

Also in agreement with the Farr et al. (25) findings of increased cycle length, an increase in follicular phase length was observed in this study. When municipal plant water data (Syngenta Crop Protection, Inc.) were used to calculate atrazine (and chlorotriazine)

estimated 'dose', there were statistically significantly associations with increased follicular phase length. According to Baird et al. (150), cycles with a lengthened follicular phase are associated with decreased probability of conception. These findings of increased cycle length are further supported by toxicological studies conducted in laboratory animals which show that atrazine exposure alters estrus cyclicity (57, 58, 59, 80, 84). Wetzel et al. (59) reported a statistically significant lengthening of the estrous cycle, in particular, an increase in the number of days in estrus in Sprague-Dawley rats. Laws et al. (58) reported irregular cycles characterized by an increase in the number of days in diestrus with atrazine exposure. An increase in the length of diestrus with atrazine exposure was also observed by Simic et al. (57).

Overall, there were no statistically significant associations between atrazine exposure and menstrual cycle length as measured with menstrual diary data. Since the latency period from atrazine exposure to menstrual cycle disruption is unknown, it is uncertain whether data collected from prospective diaries would reflect the present exposure or exposure months earlier. A menstrual diary employs a prospective assessment attempting to evaluate present atrazine concentrations with the next menstrual cycle. If the menstrual cycle diary data reflected the present atrazine concentration (the 2005 atrazine concentration) with an approximate one month latency, it is possible that atrazine concentrations were too low to result in altered menstrual cycle length. Additionally, should there be an association between atrazine and menstrual cycle disruption, it is unknown how long an effect would persist. Studies have not been done to examine how long an atrazine effect on the menstrual cycle in women would last. It is possible effects

were observed with historical questionnaire data because questionnaires represented cycles of the prior 12 months and, therefore, were reflecting exposures back to 2004 when atrazine levels were higher. If the effects of atrazine on the menstrual cycle do not persist for months or longer and atrazine concentrations were not high enough to elicit an immediate effect, then a difference in menstrual cycle length may not have been observed with prospective diary data obtained in 2005.

This study was also the first to examine potential associations between atrazine exposure through drinking water and reproductive hormone levels and the first to report effects on hormone concentrations in women with this known endocrine disruptor. The primary association found was a suppression of mid-luteal phase $E_1$3G -using several different indicators of exposure. Estrone 3-glucuronide is a major metabolite of estradiol and correlates well with circulating estradiol, the most dominant and biologically active estrogen. Estrogens were described by McLachlan et al. (210) as one of the most important hormones in women's reproduction with "the right amount at the right time and in the right place the key to its proper function."

Using state of residence as an exposure variable, an imprecise association with mid-luteal phase $E_1$3G was apparent. $E_1$3G was also associated with atrazine exposure as determined by concentrations in residential tap water. When the amount of tap water consumed was taken into consideration to estimate 'dose', this association became stronger and statistically significant ($p = 0.01$).

A decrease of mid-luteal phase $E_1$3G with atrazine exposure is in accordance with some (138, 139, 140, 211) but not all (59, 132) of the toxicological literature. The study by Coady et al. (138) found a statistically significant decrease in estrogen in African clawed frogs exposed to 0.1 ppb atrazine. Mitak et al. (140) reported a significantly lower concentration of estradiol in rats dosed with atrazine. Gojmerac et al. (139) found female pigs receiving 2 mg/kg of atrazine had statistically significantly lower estradiol concentrations than non-treated pigs. Cummings et al. (132) dosed four strains of rats with atrazine but estradiol levels were only significantly increased in the Sprague-Dawley strain dosed with 200 mg/kg of atrazine. Wetzel et al. (59) also reported increased estradiol levels in Sprague-Dawley rats fed atrazine (70 ppm and 400 ppm); no significant increases were observed in Fischer 344 rats fed the same concentrations.

Previous epidemiologic studies have provided evidence of health effects due to reduced estrogen. Since luteal estrogen stimulates the endometrium to thicken in order to create an optimal environment for implantation, a decrease in estrogen could result in implantation difficulties. Baird et al. (150) reported non-conception was associated with decreased mid-luteal phase $E_1$3G. The first chronic condition reported to be associated with estrogen deficiency was osteoporosis (212). Effects of reduced levels of circulating estrogens in older women have also been observed with cardiovascular disease (since estrogen influences lipoprotein metabolism) (213), and central nervous system deterioration (214, 215).

A reduction in $E_1$3G during the luteal phase was further supported by a reduction in Pd3G during the luteal phase. Exposure to atrazine through drinking water appeared to have an effect on the concentration of progesterone during the luteal phase. Mid-luteal phase Pd3G levels declined with increasing exposure to atrazine measured as estimated 'dose'. Since progesterone is necessary for luteal function and luteal function is needed to prepare the uterine lining for implantation, progesterone is the best indicator of luteal function (J Kesner, personal communication, November 2008). Although not statistically significant, Baird et al. (150) also observed a decrease in Pd3G among women not able to conceive. Since Pd3G is the dominant hormone secreted by the corpus lutem during the luteal phase, decreased hormone levels during the luteal phase may indicate the corpus luteum is not functioning at optimal levels. Luteal phase deficiency is a clinical diagnosis of recurring deficiency of Pd3G during the luteal phase (216). Implantation and early pregnancy can be blocked by Pd3G deficiency (216) which has been reported to be associated with infertility and repeated abortions (217). Jones et al. (218) reported an attenuated LH surge in both magnitude and duration in women with luteal phase deficiency. It has been suggested that decreased gonadotropin (LH) support of the corpus luteum can lead to luteal phase deficiency and that an inadequate LH surge can impair the corpus luteum despite the presence of a normal follicle (216). Decreased LH surge levels, although not statistically significant, were also observed in this study.

Associations between atrazine exposure and reproductive hormones were strongest and most consistent when estimated 'dose' (i.e., tap water atrazine concentration x water consumption) was used as the exposure metric. 'Dose' should be a better marker of

atrazine exposure than just concentration in tap water. Measuring the amount of unfiltered water ingested is important since certain filters remove atrazine. Furthermore, consumption patterns differ among women; therefore, an exposure metric that takes these factors into account is likely to reduce misclassification.

An attempt was made to confirm several previously established associations between phase length (luteal and follicular) and peak Pd3G in this population. The following menstrual cycle characteristics: menstrual cycle length, menstrual cycle length regularity and going > six weeks/ ≤ six weeks without a menstrual period were evaluated. As expected, follicular phase length was statistically significantly associated with cycle length (as reported by the menstrual cycle diary). In fact, a one day increase in follicular phase length was associated with an approximate one day increase in cycle length. Previous findings have established the biological variability of the menstrual cycle is the result of changes during the follicular phase, mainly due to the fluctuating timing of ovulation. It is the luteal phase of the menstrual cycle that typically remains constant (201, 219). This was consistent with findings of this study which showed luteal phase length was not associated with menstrual cycle diary length. Peak Pd3G was expected to be associated with menstrual cycle length, length regularity and going more than 6 weeks without a menstrual period; however, this was not observed. Again, the small sample size may have limited the ability to detect these associations.

Another important contribution of this study was the finding of a high overall agreement between prospective diaries and retrospective questionnaires, especially when a normal

menstrual cycle was defined as 25-35 days. These findings are consistent with those of Gold et al. (153) who showed self-report questionnaires provide reasonably accurate estimates and, although reported diary menstrual cycle lengths are more dispersed than questionnaire reported menstrual cycle lengths, the central tendency for both is similar. A high overall agreement between prospective diaries and retrospective questionnaires but low unadjusted Cohen's kappas was observed in this study. The kappa coefficients were most likely low because of high chance agreement due to differences between cells *a* and *d* of Table 3.40 (i.e., differences between 12 and 34 and differences between 6 and 50). In practical terms, the substantial prevalence effect was the result of women being more likely to report being regular on both the diary and the questionnaire than to report being irregular on both the diary and the questionnaire. Therefore, although Cohen's kappa was low, it was shown by this study that it was kept deceptively low by the high likelihood of chance agreement resulting from the high prevalence effect. The demonstration of the effect of prevalence on the kappa statistic was another contribution of this study.

Smith-DiJulio et al. (181) reported weak agreement of cycle irregularity between prospective calendars and retrospective questionnaires (Cohen's kappa = 0.19) in spite of an overall agreement of 66.9%. However, a PI of 0.48 was calculated for their data suggesting that Smith-DiJulio et al.(181) reported an artificially low kappa. Other studies reported contrary findings and suggested prospective diaries are necessary to accurately characterize cycle variations; however, two of these studies restricted their total

population to women who reported regular menstrual cycles with lengths between 21 and 35 days (175, 220, 221).

Limitations

A major overall limitation of this study was the small sample size. This is especially true for the subset of 39 women providing urine samples. The small sample size resulted in decreased precision and difficulty interpreting the results since many of the confidence intervals were wide. With small samples sizes, an outlier can strongly influence the results. Additionally, small sample sizes can lack the power necessary to detect true differences (222). Therefore, non-significant findings may not mean that no association exists, but rather the power was inadequate to detect the association (222).

Furthermore, it is possible that the results are not generalizable since women participating in an effort intensive study providing daily urine samples and/or maintaining daily menstrual diaries may not be like the rest of the population. For example, women able to participate may have more free time and, therefore, be more likely to exercise. If these women are more likely to have altered menstrual cycles from exercising more and are more likely to be exposed to atrazine, then a bias away from the null could result. However, there is no reason to believe participation was related to atrazine exposure, therefore, selection bias was unlikely. Nonetheless, the results may not be applicable to all populations since the women in this study were predominately white and middle-class with some college education.

It is also possible women with extremely irregular cycles may not have been included due to the exclusion criteria. Women taking oral contraceptives were ineligible because the pill determines bleeding and hormonal patterns. Since some women take birth control pills to regulate their menstrual cycles, menstrual cycle irregularity may be underrepresented in this study. However, Wegienka and Baird (223) found little evidence of biased estimates from not including oral contraceptive users. Women were also excluded if they had surgery on any reproductive tissue or had an endocrine related condition since these circumstances can affect hormone levels and bleeding characteristics. However, if these preexisting diseases are in the causal pathway of altered menstrual cycle characteristics either being precursors or successors of disease, eliminating these women could have underestimated risk. In addition, women who self-reported endocrine related conditions were probably unaware of subclinical disorders and, if associations with atrazine existed, again, risks could have been attenuated.

As with the use of any self-reported data from a retrospective questionnaire, the possibility of information bias exists. Although the possibility exists for misclassification of both menstrual outcome and atrazine exposure assessed via personal habits (i.e., water consumption), it is not believed women would recall differently based on their exposure or outcome status and therefore, any misclassification would be nondifferential. In addition, it is expected that the immediately preceding year is a reasonable timeframe from which monthly events can be recalled reasonably accurately.

The single menstrual cycle prospective follow-up was also a weakness of the study. It is possible a disruption of hormones from one cycle may affect the follicle development in the next menstrual cycle resulting in the potential for effects not to be seen for two or more cycles following a hormonal disruption (or exposure) (221). Small et al. (221) suggest two cycles are necessary to accurately estimate a woman's usual cycle length using prospective menstrual diaries.

It is possible findings were the result of an unmeasured chemical(s) or unmeasured exposure(s). In an effort to ensure the water systems had similar background levels of unmeasured contaminants, communities with the same type of water source (i.e., surface water) were chosen in both states. Additionally, levels of total haloacetic acids and total trihalomethanes were checked in all municipal water systems to verify levels did not exceed their respective standards. However, it is possible chlorination by-product levels below the standard are associated with in altered menstrual cycle characteristics (33), and, therefore, findings are the consequence of a difference in chlorination by-product levels. It is also possible other pesticides, for example, 2,4-dichlorophenoxyacetic acid which is used on corn, were responsible for the findings. Ideally, these would have been measured in each woman's tap water sample and controlled for in the analyses if appropriate. Another limitation of the exposure assessment was that many of the water and urine samples analyzed resulted in atrazine levels that were below the limit of detection. More samples resulting in a greater variety of concentrations, would have improved the exposure assessment.

A major limitation of this study was the lack of exposure assessment during the critical *in utero* or childhood time period when each participating woman may have been exposed to atrazine. Since the fetus and infants are particularly susceptible to adverse environmental exposures, associations could have been overlooked if the wrong exposure time period was used. Recent increased evidence emphasizes the importance of a life span approach where early exposures are assessed (224, 225). Exposure to endocrine disruptors during development has been shown to increase the incidence of reproductive abnormalities such as hypospadias, cryptorchidism, decreased fertility, accelerated puberty, and cancer (226, 227). Toxicologic studies have also shown even brief exposure during the critical time periods can increase the likelihood of cancer (228). Adverse effects in animals have been observed with prenatal exposure to endocrine disrupting compounds such as vinclozoline, bisphenol A and DES (229, 230, 231). Bibbo et al. (232) reported associations between menstrual cycle irregularities (oligomenorrhea) and women exposed *in utero* to the endocrine disruptor DES. Evidence with menstrual cycle irregularity continues to be seen in third generation women whose mothers were prenatally exposed to DES (31).

Although the women participating in this study provided information on infertility, number of pregnancies, number of deliveries and number of live births, associations with atrazine were not assessed. This study was unable to assess these potential associations because data on duration of unprotected intercourse and data on male infertility and reproductive health effects were not obtained.

Additionally, menstrual cycle abnormalities are considered common with a prevalence of 30% in the general population reported by Munster et al. (198) Therefore, the outcome for this study was not rare and, consequently, the odds ratios may not approximate the relative risks very well.

With regards to the retrospective versus prospective comparison, temporality might be of concern since participants first answered questionnaires and then completed diaries. Since the time periods of interest did not overlap, it is possible that any observed discordance was biologically accurate. However, asking a woman to answer a questionnaire after she recently maintained a diary could influence her knowledge and consequently her answers since the diary serves as a memory recall device (181). Therefore, answering a questionnaire before maintaining a menstrual diary may be more appropriate.

Finally, due to the number of statistical comparisons, the possibility that some results are due to chance cannot be excluded.

Strengths

This study had several strengths. Incorporating a prospective follow-up component allowed a temporal relationship and a direct estimate of effect to be established. This study was also able to investigate the occurrence of a dose-response relationship by evaluating objective quantitative measurements of both exposure and outcome. For exposure assessment, the Organic Analytical Toxicology Branch Laboratory of the CDC

determined atrazine levels by analyzing tap water and urinary biomarkers. In addition to being blinded as to the outcome status of the participants, atrazine samples were collected and analyzed in duplicate. Nguyen et al. (189) recently developed and validated a sensitive method that increased the number of atrazine metabolites detectable in urine. Since some of the atrazine metabolites are considered equal in toxicity to their parent compound, expanding the list of metabolites provides a better representation and more accurate assessment of atrazine exposure and ultimately atrazine toxicity. This methodology was used by the CDC laboratory for this study.

For outcome evaluation, reproductive hormones were also objectively assessed. In order to evaluate reproductive hormone secretion, modified fluoroimmunoassays developed and validated by the NIOSH Reproductive Endocrinology Laboratory to measure urinary LH, $E_1$3G, and Pd3G were used (192, 193). These assays provide accurate, sensitive, and specific assessment of reproductive hormones. In addition, the NIOSH laboratory was also blinded to the exposure status of each participant.

The presence of an association between atrazine and estrogen and an association between atrazine and progesterone fits well with biological processes of the hypothalamic-pituitary-ovarian axis and offers a plausible mechanism of action. Furthermore, the associations identified in this study are compatible with previous toxicological findings and, although limited, epidemiologic investigations. Most studies have shown atrazine does not have intrinsic estrogenic activity and is unable to bind to the estrogen receptor (82, 84, 233). In fact, some toxicological findings indicate atrazine may be anti-

estrogenic (82, 84). While this study did not evaluate whether atrazine binds to the estrogen receptor or not, it did show a suppression of estrogen concentrations possibly indicating atrazine exposure is anti-estrogenic.

Another strength of this study was that in an attempt to assess any mixing of effects leading to possible biases, many potential confounders were considered. Based on past epidemiologic literature and the risk factors associated with menstrual cycle regularity, a comprehensive list of potential confounding variables was developed. The list included: age, BMI, parity, current smoking status, weekly alcohol consumption, education, age at menarche, caffeine consumption, vegetable consumption, fruit consumption, income, and physical activity. Confounding was assessed and controlled for in the statistical analyses where appropriate. However, it is possible that residual confounding still exists.

Finally, all information was collected by a single trained interviewer.

Conclusions

Even though the majority of atrazine concentrations in this study were below the EPA standard, exposure to atrazine in municipal drinking water was associated with altered menstrual cycles. Length irregularity, increased follicular phase length and increased cycle length were significantly associated with atrazine exposure. Moreover, the reproductive hormone results provided further support of the menstrual cycle findings and offer the possibility of reduced fecundability in women exposed to atrazine. Given the dependence of reproductive hormones on one another in this feedback loop system,

modifications in any hormone level are expected to lead to a vicious cycle of endocrine changes and in, due course, menstrual irregularities (146).

Accompanying the atrazine exposure findings, results of this study indicated questionnaire data may be more reliable than previously reported. This study suggests that when a normal menstrual cycle is defined as every 25 to 35 days, doubts about retrospective questionnaires may be unjustified. Although Cohen's kappa was low, it was shown to have been deceptively low by the high likelihood of chance agreement resulting from the high prevalence effect. The demonstration of the effect of prevalence on the kappa statistic was another contribution of this study.

It is unknown whether atrazine exposure affects fecundity in humans but findings of hormone changes and altered menstrual cycle characteristics suggest the possibility exists. Results of this study are preliminary and further studies, on larger populations, are needed to confirm the findings. Associations with other reproductive disorders, and conditions such as osteoporosis, cardiovascular disease and birth defects should be explored. Future studies should also be pursued in men to examine the effects of exposure on semen quality, and in persons exposed to higher concentrations of atrazine, as well as populations exposed to other hormonally active pesticides, such as lindane and mancozeb. Future studies are also needed to more accurately define the shape of the dose-response curve and to explore additional hormonal pathways in detail.

While these data are preliminary, precautionary efforts should be taken to reduce atrazine drinking water exposure. Riparian buffers of at least 60 feet in width should be established surrounding surface drinking water sources in order to filter pollutants such as atrazine and protect waters from agricultural run-off (234). The results of this study combined with future work could have regulatory implications for atrazine exposure in drinking water. Given the findings of this study and the fact that atrazine is a known endocrine disruptor, it is possible the current EPA MCL is not protective of human health. The EPA should continue to evaluate new research as it becomes available and reconsider the current MCL based on new findings. Should these findings be confirmed and a new MCL established, activated carbon filtration systems to remove atrazine should be installed at municipal plants exceeding the new MCL and a moratorium on atrazine application in the watersheds of these plants should be implemented.

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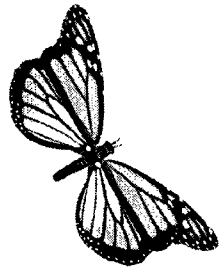
7. Appendices

Appendix A. Questionnaire



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Menstrual Cycle Characteristics and Reproductive Patterns in Women Exposed to Atrazine in Drinking Water

Personal Data Sheet

CSU Identification Number_____

NIOSH KIT #_____ **Place CDC ID "Q" Label here:**

----------Please print----------

Date:_____

Participant Name:_____

Home Address: _____

City: _____ **State:** _____ **Zip:** _____

Home Phone: _____

Work Phone: _____

Social Security Number:_____
(required for payment by CSU)

We know that some of these questions may be sensitive to you. I want to reassure you that your answers are important to us and that all information from this interview will be held strictly confidential and not used in any way that can identify you. Your name or information will not be released to any private party, employer or insurance company. Additionally, you can refuse to answer any question with which you don't feel comfortable answering.

Menstrual Cycle Characteristics and Reproductive Patterns in Women Exposed to Atrazine in Drinking Water Questionnaire

CSU Identification Number: _____(to be completed by CSU investigator)

DEMOGRAPHICS

1) What is your date of birth? Month_____ Day_____ Year_____

2) How old are you? _____years

3) How much do you weigh? _____pounds or _____kg

4) How tall are you? _____feet _____inches or _____cm

5) Which of the following groups best describes your race?
   1. White
   2. Black, African American
   3. Asian/Pacific Islander
   4. American Indian/Alaskan native
   5. Other (Specify)_____
   6. Refuse

6) Are you of Hispanic origin?
   1. Yes 2. No 99. Don't Know

7) What is the highest grade of school that you have completed?
   1. No formal schooling
   2. Grades 1-8 (elementary school)
   3. Grades 9-11 (some high school)
   4. High School or GED
   5. High School + Technical Vocational Training
   6. Some College or Associate's degree
   7. Bachelor's degree
   8. Master's degree
   9. Doctorate

8) Which number in the following list comes closest to the total income for your family in 2004? Include the income of all family members who live in the household.
   1. under $15,000
   2. $15,000 - $29,999
   3. $30,000 - $44,999
   4. $45,000 - $59,999
   5. $60,000 - $74,999
   6. $75,000 - $99,999
   7. 100,000 – 114,999
   8. 115,000 - $129,999
   9. Over 130,000
   10. Refuse
   99. Don't know

WORK/OCCUPATION:

9) What is your present job title? _____

10) How long have you worked at the job you entered above?_____years

11) Do you work on a farm or in agriculture?
1. Yes 2. No

12) Do you have contact with any pesticides/herbicides at your job?
1. Yes 2. No 99. Don't Know

13) How many years have you lived in your current home? _____

14) Do you live next door or across the road from a farm? 1. Yes 2. No 99. Don't Know

15) Are there any farms within 1 mile from your house? 1. Yes 2. No 99. Don't Know

16) Were chemicals such as weed killers, termite control, insecticides, or pesticides ever used around your home or family gardens?
1. Yes 2. No 99. Don't Know

17) How many days per year did you personally handle these pesticides?
DAYS/YR _____ 2.NONE 99. Don't Know

18) What pesticides were used around the home or family gardens? RECORD NAME(S).

1. _____
2. _____
3. _____
4. _____
5. _____
99. Don't Know

ALCOHOL AND SMOKING HISTORY

19) In a typical month, how often did you usually drink any kind of alcoholic beverage?
1. Never (Go to question # 21)
2. Less than one time a month
3. 1 time a week
4. 2-4 times a week
5. Almost every day
6. Every day

20) On a typical day when you drink, about how many drinks do you consume? (Note: A drink is 1 can or bottle of beer, or 1 glass of wine or wine cooler, or 1 cocktail, or 1 shot of liquor.)

Number of drinks: _____ (Enter zero if none)

The next questions are about your smoking habits.

21) During your entire lifetime, have you smoked a total of 5 packs or more?(1 pack = 20 cigarettes)?
1. YES 2. NO 99. Don't Know

22) Have you smoked cigarettes in the past year?
1. YES 2. NO 99. Don't Know

23) How many years in total (did you smoke/have you been smoking)?
_____# years

24) On average over the past 6 months how many cigarettes did you usually smoke per day?
_____# cigarettes

25) Was there ever a time when you QUIT SMOKING for a year or more and then started again?
1. YES 2. NO

a. For how many years did you quit altogether? _____# years

26) On the average during the whole time you (smoked/have been smoking), about how many cigarettes did you smoke each day?
_____# cigarettes

DIETARY INFORMATION

27) How many cups, glasses or cans of caffeinated beverages do you drink on a typical day? (Enter 0 if none)

1. Cup of Coffee: _____
2. Cup of Tea (Hot or Iced): _____
3. Cup of Chocolate (Cocoa): _____
4. Can of Caffeinated Soda Pop (Soft drinks examples: Coke, Pepsi, Diet Coke, Diet Pepsi, Mello Yello, Sunkist Orange Mountain Dew, Dr. Pepper): _____
5. Others: _____ (Specify: _____)
99. Don't know

28) How often do you eat vegetables (salads, vegetable juice, etc.) on a typical day?
(Note: 1 serving is 1 raw vegetable or enough prepared vegetables to fit into the palm of your hand.)

0. Never
1. 1 serving per day
2. 2 servings per day
3. 3 servings per day
4. 4 servings per day
5. 5 servings per day
6. Occasionally (Less than 1 serving per day)

29) How often do you eat fruits (including fruit juice) on a typical day?
(Note: 1 serving is 1 raw fruit or enough prepared fruit to fit into the palm of your hand.)

0. Never
1. 1 serving per day
2. 2 servings per day
3. 3 servings per day
4. 4 servings per day
5. 5 servings per day
6. Occasionally (Less than 1 serving per day)

30) Do you take any supplements (vitamins, minerals, or herbals)? 1. Yes 2. No 99. Don't Know

31) Which supplements?_____

32) How often (daily or several times per week)?_____

PHYSICAL ACTIVITY

33) During a typical week, how many hours do you spend doing strenuous exercise (heart beats rapidly)?

a) In the summer:

1. None
2. Less than 1 hour
3. 1-2 hours
4. 3-5 hours
5. 6-10 hours
6. More than 10 hours

b) In the winter:

1. None
2. Less than 1 hour
3. 1-2 hours
4. 3-5 hours
5. 6-10 hours
6. More than 10 hours

WATER SOURCE

34) To whom do you pay your water bill? _____ 99. Don't Know

35) Is the water you usually use for <u>drinking</u> filtered?
1. Yes (see question # 36) 2. No (skip to question # 39) 99. Don't Know

36) What type of filtration system is used?
1. Membrane filter
2. Charcoal filter
3. Other _____
99. Don't know

37) What is the brand name of the treatment/filtration system?_____

38) What is the model number or type of the filtration system? _____

39) Is the water you usually use for <u>cooking</u> filtered?
1. Yes (see question # 40) 2. No (skip to question # 43) 99. Don't Know

40) What type of filtration system?
1. Membrane filter
2. Carbon/Charcoal filter
3. Other _____
99. Don't know

41) What is the brand name of the treatment/filtration system?_____

42) What is the model number of the filtration system? _____

WATER CONSUMPTION (AT HOME)

The next questions will ask about your water consumption at home.

43) What is the usual source of water you use for drinking at home?
1. unfiltered tap water 2. filtered tap water 3. bottled water 4. other (please specify)_____

44) During a typical day while at home, how many glasses of plain water do you drink at home?
_____glasses unfiltered tap water
_____glasses filtered tap water
_____glasses bottled water
_____glasses other water (please specify)_____
99. Don't Know

45) During a typical day while at home, how many glasses of powdered or concentrate water do you drink (for example, Kool-Aid, iced tea or lemonade) at home?
_____glasses unfiltered tap water
_____glasses filtered tap water
_____glasses bottled water
_____glasses other water (please specify)_____
99. Don't Know

46) During a typical day while at home, how many glasses of hot drinks made with water do you drink (for example, coffee or tea) at home?
_____glasses unfiltered tap water
_____glasses filtered tap water
_____glasses bottled water
_____glasses other water (please specify)_____
99. Don't Know

47) What is the usual source of water you use for cooking and food preparation at home?
1. unfiltered tap water
2. filtered tap water
3. bottled water
4. other water (please specify)_____
99. Don't Know

WATER CONSUMPTION (AT WORK OR AT SCHOOL)

The next questions will ask about your water consumption at work or at school.

48) What is the usual source of water you use for drinking at work or school?
1. unfiltered tap water 2. filtered tap water 3. bottled water 4. other (please specify)_____

49) If you store water for drinking, what is the storage container made out of?
1. Plastic 2. Metal 3. Glass 4. other (please specify)_____ 99. Don't store

50) During a typical day while at home, how many glasses of plain water do you drink at work/ or at school?
_____glasses unfiltered tap water
_____glasses filtered tap water

_____glasses bottled water
_____glasses other water (please specify)_____
99. Don't Know

51) During a typical day while at home, how many glasses of powdered or concentrate water do you drink (for example, Kool-Aid, iced tea or lemonade) at work or at school?
_____glasses unfiltered tap water
_____glasses filtered tap water
_____glasses bottled water
_____glasses other water (please specify)_____
99. Don't Know

52) During a typical day while at home, how many glasses of hot drinks made with water do you drink (for example, coffee or tea) at work or at school?
_____glasses unfiltered tap water
_____glasses filtered tap water
_____glasses bottled water
_____glasses other water (please specify)_____
99. Don't Know

MENSTRUAL HISTORY
The next set of questions ask about the length of your menstrual cycles.

53) How old were you when you had your first menstrual period? 1. _____(age in years)
2. Never
99. Don't Know

54) On what date did your most recent menstrual period start? (mm/dd/yy)_____ 99. Don't Know

55) What is the expected date of your next menstrual period? (mm/dd/yy)_____99. Don't Know

56) Generally speaking, are your periods regular or irregular? That is, is the length of time between the first day of one period and the first day of the other about the same each cycle?
1.Yes 2.No 99. Don't Know

57) Can you predict the onset of your period within 4 days by using the calendar (without using premenstrual symptoms you may have)?
1. Yes 2. No 99. Don't Know

58) Many women have their periods about once a month. Some women have their periods more often and others less often. How often are your menstrual periods? In other words, how many days are there from the first day of one menstrual period to the first day of the next period?

1. 24 days or less
2. 25-30 days
3. 31-35 days
4. 36-42 days
5. 43 days or more
6. Too irregular to say
99. Don't Know

59) What is the LONGEST menstrual cycle you've had in the last 12 months? Count from the first day of one period to the first day of the next.

_____ days 99. Don't Know

60) In what season did this occur? (Mark all that apply)

1. Summer 2. Winter 99. Don't Know
3. Fall 4. Spring

61) What is the SHORTEST menstrual cycle you've had in the last 12 months? Count from the first day of one period to the first day of the next.

_____ days 99. Don't Know

62) In what season did this occur? (Circle"Yes" or "No" for each month)

Summer - Yes / No Winter - Yes / No 99. Don't Know
Fall - Yes / No Spring - Yes / No

63) During the last 12 months did you ever bleed or spot between menstrual periods (Do not count times when you were pregnant, breast feeding or using birth control pills/medication)?

1. Yes **(see next question)** 2. No **(see question #63)** 99. Don't Know

64) In what season did this occur? (CircleYes or No for each month)

Summer - Yes / No Winter - Yes / No 99. Don't Know
Fall - Yes / No Spring - Yes / No

65) During the last 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do not count times when you were pregnant, breastfeeding or using birth control pills.

1. Yes 2. No

66) Approximately, how often do you have cramps or backache with your menstrual periods?

1. Never 2. Sometimes 3. Often 4. Always 99. Don't Know

67) When you have menstrual cramps or backache, how would you describe your pain?

1. Mild = Your daily activities are not usually affected and pain medication is rarely needed.
2. Moderate = Your daily activities may be affected, pain medication is often needed and usually relieves your pain.
3. Severe = Your daily activities are definitely affected. Pain medication is needed but often does not relieve your pain.

PREGNANCY QUESTIONS

The next questions will be about pregnancies you may have had.

68) Have you and your partner ever tried to become pregnant for at least 1 year, but were unable to do so?

1. Yes, when I was ____ years old; Calendar year: _____
2. We tried to become pregnant and conceived within less than 1 year
3. Don't know

69) Have you ever been pregnant? Count all pregnancies including live births, pregnancies that ended in still births, miscarriages, or abortions.

1. Yes **(See next question)**
2. No **(Thank you, you have completed the Questionnaire)**

70) What is the total number of times that you have been pregnant?
_____ total pregnancies 99. Don't know

71) Of all your pregnancies, how many resulted in live births? _____ total pregnancies 99. Don't know

Please provide the outcome of each of your pregnancies and give the approximate date the pregnancy ended (if it was a live birth, this is the birth day of your child) and indicate how long it took you to conceive this pregnancy:

1st Pregnancy:	2nd Pregnancy:	3rd Pregnancy:	4th Pregnancy:	5th Pregnancy:	6th Pregnancy:
Pregnancy ended:	Pregnancy ended:	Pregnancy ended:	Pregnancy ended:	Pregnancy ended:	Pregnancy ended:
Month____Year____	Month____ Year____	Month____ Year____	Month____ Year____	Month____ Year____	Month____ Year____
[99] Don't Know	[99] Don't Know	[99] Don't Know	[99] Don't Know	[99] Don't Know	[99] Don't Know
[1] Normal birth Including C-section	[1] Normal birth Including C-section	[1] Normal birth Including C-section	[1] Normal birth Including C-section	[1] Normal birth Including C-section	[1] Normal birth Including C-section
[2] Stillborn	[2] Stillborn	[2] Stillborn	[2] Stillborn	[2] Stillborn	[2] Stillborn
[3] Miscarriage	[3] Miscarriage	[3] Miscarriage	[3] Miscarriage	[3] Miscarriage	[3] Miscarriage
[4] Tubal/ectopic pregnancy	[4] Tubal/ectopic pregnancy	[4] Tubal/ectopic pregnancy	[4] Tubal/ectopic pregnancy	[4] Tubal/ectopic pregnancy	[4] Tubal/ectopic pregnancy
[5] Elective abortion	[5] Elective abortion	[5] Elective abortion	[5] Elective abortion	[5] Elective abortion	[5] Elective abortion
[6] Other, specify:	[6] Other, specify:	[6] Other, specify:	[6] Other, specify:	[6] Other, specify:	[6] Other, specify:
_____	_____	_____	_____	_____	_____
How many months was it from the time you first began trying to become pregnant until you conceived? _____**months**	How many months was it from the time you first began trying to become pregnant until you conceived? _____**months**	How many months was it from the time you first began trying to become pregnant until you conceived? _____**months**	How many months was it from the time you first began trying to become pregnant until you conceived? _____**months**	How many months was it from the time you first began trying to become pregnant until you conceived? _____**months**	How many months was it from the time you first began trying to become pregnant until you conceived? _____**months**
99. Don't Know	99. Don't Know	99. Don't Know	99. Don't Know	99. Don't Know	99. Don't Know
Would you say that you are quite sure about this, relatively sure, or unsure.	Would you say that you are quite sure about this, relatively sure, or unsure.	Would you say that you are quite sure about this, relatively sure, or unsure.	Would you say that you are quite sure about this, relatively sure, or unsure.	Would you say that you are quite sure about this, relatively sure, or unsure.	Would you say that you are quite sure about this, relatively sure, or unsure.
[1] Quite sure [2] Relatively sure [3] Unsure	[1] Quite sure [2] Relatively sure [3] Unsure	[1] Quite sure [2] Relatively sure [3] Unsure	[1] Quite sure [2] Relatively sure [3] Unsure	[1] Quite sure [2] Relatively sure [3] Unsure	[1] Quite sure [2] Relatively sure [3] Unsure

Please fill out for each pregnancy

Pregnancy	Were you living at your current address at the time of pregnancy?		Child's Date of birth: (mm / yy)	Child's Weight at birth:	Child's Gender:		Length of pregnancy:	Did the doctor tell you the baby: had low birth weight?		was premature?	
1st	Yes	No; If you lived elsewhere in Mt. Olive at that time please give address _____	(____/____)	___lbs ___oz 99. Don't Know	M [1]	F [2]	____ wks	Yes [1]	No [2] 99. Don't Know	Yes [1]	No [2] 99. Don't Know
2nd	Yes	No; If you lived elsewhere in Mt. Olive at that time please give address _____	(____/____)	___lbs ___oz 99. Don't Know	M [1]	F [2]	____ wks	Yes [1]	No [2] 99. Don't Know	Yes [1]	No [2] 99. Don't Know
3rd:	Yes	No; If you lived elsewhere in Mt. Olive at that time please give address _____	(____/____)	___lbs ___oz 99. Don't Know	M [1]	F [2]	____ wks	Yes [1]	No [2] 99. Don't Know	Yes [1]	No [2] 99. Don't Know
4th	Yes	No; If you lived elsewhere in Mt. Olive at that time please give address _____	(____/____)	___lbs ___oz 99. Don't Know	M [1]	F [2]	____ wks	Yes [1]	No [2] 99. Don't Know	Yes [1]	No [2] 99. Don't Know
5th	Yes	No; If you lived elsewhere in Mt. Olive at that time please give address _____	(____/____)	___lbs ___oz 99. Don't Know	M [1]	F [2]	____ wks	Yes [1]	No [2] 99. Don't Know	Yes [1]	No [2] 99. Don't Know

Do you have any additional comments you would like to share?

_____

_____

_____

_____

_____

You are now finished with the questionnaire – Thank you!

Appendix B. Acronyms, abbreviations and symbols

AM	atrazine mercapturate
AZN	atrazine
AZN-OH	hydroxyatrazine
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CNS	central nervous system
Cr	creatinine
CSU	Colorado State University
CWS	community water system
cyclic AMP	cyclic adenosine monophosphate
CYP	cytochrome
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyl trichloroethene
DEA	desethylatrazine
DEAM	desethylatrazine mercapturate
DES	diethylstilbestrol
DLT	day of luteal transition
E13G	estrone 3-glucuronide
EPA	Environmental Protection Agency
FR	fecundability ratio
FSH	follicle stimulating hormone
GC/MS	gas chromatographic/mass selective detection
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin
HPLC-MS/MS	high performance liquid chromatography-tandem mass spectrometry
HR	hazards ratio
IA	immunoassay
IRED	interim reregistration eligibility decision
IUD	intrauterine device
IUGR	intrauterine growth retardation
LC/MS	liquid chromatographic/mass spectrometry/mass spectrometry detection
LH	luteinizing hormone
MCL	maximum contaminant level
mg	milligram
mIU	milli international units
n	number
ng	nanogram
NIOSH	National Institute for Occupational Safety and Health
OR	odds ratio
p	p-value

PCB	polychlorinated biphenyl
Pd3G	pregnanediol 3-glucuronide
PI	prevalence index
POC	persistent organochlorine compound
ppb	parts per billion
ppm	parts per million
PSA	prostate specific antigen
RR	risk ratio
SAS	statistical analysis software
SD	standard deviation
SF-1	steroidogenic factor 1
SOC	synthetic organic chemical
TCDD	tetrachlorodibenzodioxin

$>$	greater than
$\geq$	greater than or equal to
$=$	equal to
$<$	less than
$\leq$	less than or equal to
%	percent
β	beta coefficient
α	alpha
μg	micrograms

Appendix C. Urinary Reproductive Hormone and Phase Length Algorithms

LH peak
Highest value of the cycle that exceeds 8.5 mIU LH/mg CR. (Omit if there is missing data on day adjacent to highest value; if highest value is not 4 days after a rise >2.5-fold above the mean of the previous 7 days with no more than 3 missing days; if the cycle is without a start menstrual period and does not have 17 days of sampling; if the cycle is without an end menstrual period and does not have 20 days of sampling; if cycle has <35 days and no menstrual period. (mIU LH/mg)

* Preovulatory LH level
Geometric mean for the 3 days ending on DLT (day of luteal transition) or day of LH surge onset. Omit if any days are missing. (mIU LH/mg)

* Mid-luteal phase $E_1$3G level
Geometric mean for days 5 & 6 after DLT or after day of LH surge onset. Omit if any missing values. (ng/mg)

* Follicular Phase Pd3G level
Geometric mean from cycle day 5 thru 3rd day before DLT or day of LH surge onset, or days 6-10. Omit if < 2 values present. Must have start menses. (μg/mg)

* Mid-luteal phase Pd3G level
Geometric mean for days 5 & 6 after DLT or day of LH surge onset. Omit if any missing values. (μg/mg)

* Follicular phase length
Day of LH surge onset or DLT (day of luteal transition). Equals luteal day 0. Must have start menses. (days)

Luteal phase length
Last day of cycle minus day after LH surge onset or after DLT. Must have end menses. (days)

* Associated with infertile ovulatory cycles according to Baird et al. 1999.