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Menstrual cycle characteristics and reproductive hormone levels in women exposed to atrazine in drinking water ☆

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ABSTRACT

Atrazine is the most commonly used herbicide in the U.S. and a wide-spread groundwater contaminant. Epidemiologic and laboratory evidence exists that atrazine disrupts reproductive health and hormone secretion. We examined the relationship between exposure to atrazine in drinking water and menstrual cycle function including reproductive hormone levels.

Women 18–40 years old residing in agricultural communities where atrazine is used extensively (Illinois) and sparingly (Vermont) answered a questionnaire ($n=102$), maintained menstrual cycle diaries ($n=67$), and provided daily urine samples for analyses of luteinizing hormone (LH), and estradiol and progesterone metabolites ($n=35$). Markers of exposures included state of residence, atrazine and chlorotriazine concentrations in tap water, municipal water and urine, and estimated dose from water consumption.

Women who lived in Illinois were more likely to report menstrual cycle length irregularity (odds ratio (OR)=4.69; 95% confidence interval (CI): 1.58–13.95) and more than 6 weeks between periods (OR=6.16; 95% CI: 1.29–29.38) than those who lived in Vermont. Consumption of >2 cups of unfiltered Illinois water daily was associated with increased risk of irregular periods (OR=5.73; 95% CI: 1.58–20.77). Estimated "dose" of atrazine and chlorotriazine from tap water was inversely related to mean mid-luteal estradiol metabolite. Atrazine "dose" from municipal concentrations was directly related to follicular phase length and inversely related to mean mid-luteal progesterone metabolite levels.

We present preliminary evidence that atrazine exposure, at levels below the US EPA MCL, is associated with increased menstrual cycle irregularity, longer follicular phases, and decreased levels of menstrual cycle endocrine biomarkers of infertile ovulatory cycles.

1. Introduction

Atrazine, a triazine herbicide applied to a variety of crops for weed control, is the most commonly used herbicide in the United States (U.S. Environmental Protection Agency, 2008) and a frequently detected contaminant in surface and drinking water in high use areas (US Environmental Protection Agency, 2003).

Concerns regarding potential health effects of exposure to atrazine in humans are based in part on reported adverse neuroendocrine and reproductive effects and the disruption of reproductive hormones including inhibition of luteinizing hormone (LH) release in laboratory animals (Cooper et al., 1996, 2007, 2000; Gojmerac et al., 2004; McMullin et al., 2004; Narotsky et al., 2001; Trentacoste et al., 2001).

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In toxicological studies, exposure to chlorinated triazines, which include atrazine and its metabolites has been shown to cause altered estrous cycles, delayed puberty, pregnancy loss, prostate inflammation, hermaphroditism and gonadal dysgenesis (Cooper et al., 2007; Hayes et al., 2002, 2003; Laws et al., 2000; Narotsky et al., 2001; Stevens et al., 1994; Stoker et al., 1999; Wetzel et al., 1994).

In humans, exposure to atrazine has been associated with intrauterine growth retardation (IUGR) (Munger et al., 1997), small-for-gestational-age (SGA) births (Ochoa-Acuna et al., 2009; Villanueva et al., 2005), spontaneous abortion (Arbuckle et al., 2001) and reduced semen quality (Swan, 2006). In the Agricultural Health Study, women who reported using pesticides, including atrazine, had an increased risk of missed periods and intermenstrual bleeding; those who mixed or applied atrazine or lindane reported longer menstrual cycles (Farr et al., 2004). Menstrual cycle characteristics, including the underlying endocrine axis, have implications not only as biomarkers of fertility issues (e.g., fecundity, spontaneous abortions, endometriosis, uterine fibroids), but are also associated with heightened risk to hormonally sensitive diseases (e.g., cancers, osteoporosis, cardiovascular disease and diabetes) (Charkoudian and Joyner, 2004; Deroo and Korach, 2006; Shuster et al., 2008, 2010; Xiao et al., 2006).

Thus, our goal was to explore the relationship between exposure to atrazine in drinking water and human menstrual cycle function, including menstrual cycle characteristics and associated hormone levels.

2. Materials and methods

2.1. Participant selection

The Illinois communities of Mount Olive and Gillespie had among the highest atrazine municipal drinking water concentrations in the nation in 2003 (Atrazine Monitoring Program, Syngenta Crop Protection, Inc.) and were selected for study as a high-exposure population. Raw water concentrations of atrazine and chlorotriazine in 2003, respectively, were 18.8 and 20.6 μg/L in Mount Olive and 5.1 and 7.2 μg/L in Gillespie. Waterbury and Fair Haven, Vermont, were selected as a low-exposure population. Vermont is an agricultural state where small amounts of atrazine are used.

The municipal offices of Mount Olive, Gillespie, Fair Haven and Waterbury provided contact information (name, address and phone number) for all residences served by their respective water utility. Each residence was sent a letter explaining the study design, data collection procedures and informed consent procedure. Subsequently, the investigator telephoned to determine whether an eligible woman lived at the residence and, if so, whether she was willing to participate. Potential participants were informed that the study focused on pesticides and reproductive health.

Participants were premenopausal women 18–40 years old residing in one of these communities in 2005. Women were not eligible to participate if they had taken any form of hormonal contraception, medication or replacement, used an intrauterine device, breast fed within the past 3 months or were pregnant within the past 6 months. Women who had been diagnosed with disorders known to affect reproduction or endocrine function were also ineligible. No eligible participants resided at 1022 (82.42%) of the homes called.

All participants ($n=102$) answered a questionnaire that included reproductive history, information on potential confounders, exposure indices and menstrual cycle characteristics. A subset of these women ($n=67$) maintained a daily diary of vaginal bleeding for one complete menstrual cycle and provided paired urine and home tap water samples on the same day. Thirty-five of these women also collected daily first-morning urine samples for a complete cycle. Each woman chose the extent of her participation. Informed consent was obtained following guidelines established by the Colorado State University Human Subjects Research Committee.

2.2. Exposure assessment

Markers of atrazine exposure, including state of residence, years in current home, and consumption of unfiltered water, were obtained from questionnaires. The daily volume of unfiltered tap water ingested, including drinks made with tap water, was calculated for each participant.

Two home tap water samples and two urine samples were collected two days apart to further assess exposure. Cold tap water was collected in pre-washed amber glass bottles with Teflon screen caps (US Environmental Protection Agency, 1994) after running the system for two minutes. Sodium sulfite (4–5 mg) was added to each water sample to remove residual chlorine (US Environmental Protection Agency, 1994). Urine samples were collected in prewashed bottles (US Environmental Protection Agency, 1994). Tap water and urine samples were placed on ice during transport to the field station, frozen at −20 °C and shipped on dry ice by next-day courier to the Centers for Disease Control and Prevention (CDC) for analysis.

Tap water and urine samples were analyzed for atrazine, the chlorotriazines desethylatrazine, desisopropyl atrazine, and diaminochlorotriazine and the atrazine metabolites atrazine mercapturate and desethylatrazine mercapturate. Samples were analyzed using online solid phase extraction coupled with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Panuwet et al., 2008). The limit of detection (LOD) was 0.5 μg/L for atrazine, atrazine mercapturate, and desethylatrazine mercapturate and 1.0 μg/L for desisopropylatrazine, desethylatrazine and diaminochlorotriazine. Urinary atrazine and metabolite concentrations were adjusted for creatinine concentration. Undetectable concentrations for atrazine and metabolites were set to the LOD divided by $\sqrt{2}$ (Hornung and Reed, 1990). Analyses for residential tap water analytes and urinary atrazine and atrazine metabolites were conducted by averaging their paired measurements and dichotomizing them at their LOD divided by $\sqrt{2}$. The estimated 'doses' for atrazine and chlorotriazines were defined as the product of the volume of unfiltered water ingested per day times the concentration of each analyte in the municipal and tap drinking water.

Levels of atrazine, desisopropylatrazine, desethylatrazine and diaminochlorotriazine were monitored at the Gillespie and Mount Olive municipal water plants by Syngenta Crop Protection, Inc. (Wilmington, DE) as mandated (U.S. Environmental Protection Agency, 2006). Municipal water in these Illinois communities was monitored weekly from April through July and once every two weeks from August through March of 2003–2007. Prior to June 2005, municipal water samples were analyzed by Syngenta using enzyme immunoassay (EIA), and samples containing more than 3.0 μg/L analyte were typically reanalyzed using gas chromatographic/mass selective detection (GC/MS). After June 2005, municipal water samples were analyzed using HPLC-MS/MS (B. Christensen, Syngenta Crop Protection, Inc., personal communication to Illinois Environmental Protection Agency (EPA), February, 2009). Results were reported as concentrations of atrazine and total chlorotriazines (sum of atrazine, desisopropylatrazine, desethylatrazine and diaminochlorotriazine). The limits of quantitation ranged from 0.05 to 0.50 μg/L (Merritt, 2006). The municipal water suppliers of Waterbury and Fair Haven, Vermont, have waivers from the state for monitoring synthetic organic chemicals (SOCs), including atrazine. Waivers are issued when SOCs have never been detected in a water supply and continue as long as no changes in land use in the source protection area occur (J Siriano, personal communication, March 2006). Municipal monitoring data for atrazine and chlorotriazines from the Mount Olive and Gillespie, Illinois, municipal water plants were also used as individual exposure variables. Monitoring data were typically unavailable for each woman's exact date of participation. Therefore, the two municipal plant results that bracketed the participation date were averaged and weighted according to the number of days from that date. The final imputed values were then multiplied by the volume of unfiltered tap water ingested per day to calculate their estimated 'dose' from this source and dichotomized using a median split for analysis.

2.3. Outcome assessment

Questionnaire data were used to assess menstrual cycle length irregularity by asking "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?". Severe length irregularity was assessed by asking participants if "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 categorized by questionnaire respondent as ≤ 24 days, 25–30 days, 31–35 days, 36–42 days and ≥ 43 days (Farr et al., 2004). For statistical analysis, menstrual cycle length was further dichotomized as ≤ 30 days and > 30 days. Intermenstrual bleeding and dysmenorrhea were also assessed.

Menstrual cycle length was also assessed using data from the prospective menstrual cycle diaries in which women ($n=67$) recorded the absence or presence and relative amount of bleeding through two complete menstrual bleeding periods.

A subset of participants ($n=35$) collected daily first morning urine voids for reproductive endocrine assessments beginning the day after their entry interview and continued through the third day after the end of their second study bleeding period. Urine samples were stored in polypropylene vials with glycerol (7% final dilution) to prevent loss of gonadotropin activity when frozen (Kesner et al., 1995). Participants stored urine samples in their home freezer. At the end of collection, they shipped the frozen urine samples on ice packs in Styrofoam to the National Institute for Occupational Safety and Health (NIOSH) Reproductive Endocrinology Laboratory by next-day courier at the end of collection. Samples were stored at NIOSH at −80 °C until analyzed (Kesner et al., 1995).