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In utero exposure to atrazine analytes and early menarche in the Avon Longitudinal Study of Parents and Children Cohort

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ABSTRACT

Background: Evidence from experimental studies suggests that atrazine and its analytes alter the timing of puberty in laboratory animals. Such associations have not been investigated in humans.
Objective: To determine the association between in utero exposure to atrazine analytes and earlier menarche attainment in a nested case-control study of the population-based Avon Longitudinal Study of Parents and Children.
Methods: Cases were girls who reported menarche before 11.5 years while controls were girls who reported menarche at or after 11.5 years. Seven atrazine analyte concentrations were measured in maternal gestational urine samples (sample gestation week median (IQR): 12 (8–17)) during the period 1991–1992, for 174 cases and 195 controls using high performance liquid chromatography-tandem mass spectrometry. We evaluated the study association using multivariate logistic regression, adjusting for potential confounders. We used multiple imputation to impute missing confounder data for 29% of the study participants.
Results: Diaminochlorotriazine (DACT) was the most frequently detected analyte (58% > limit of detection [LOD]) followed by desethyl atrazine (6%), desethyl atrazine mercapturate (3%), atrazine mercapturate (1%), hydroxyl atrazine (1%), atrazine (1%) and desisopropyl atrazine (0.5%). Because of low detection of other analytes, only DACT was included in the exposure–outcome analyses. The adjusted odds of early menarche for girls with DACT exposures ≥ median was 1.13 (95% Confidence Interval [95% CI]:0.82, 1.55) and exposure < median was 1.01 (95% CI: 0.73, 1.42) compared to girls with exposure < LOD (reference). In the subset that excluded girls with missing data, the adjusted odds of early menarche for girls with DACT exposures ≥ median was 1.86 (95% CI: 1.03, 3.38) and exposure < median was 1.26 (95% CI: 0.65, 2.24) compared to the reference.
Conclusions: This study is the first to examine the association between timing of menarche and atrazine analytes. We found a weak, non-significant association between *in-utero* exposure to atrazine metabolite DACT and early menarche, though the association was significant in the subset of girls with complete confounder information. Further exploration of the role of these exposures in female reproduction in other cohorts is needed.

1. Introduction

Atrazine is used in more than 70 countries worldwide; most frequently in the United States (U.S.), Brazil, Argentina, Mexico and China to control broadleaf and grassy weeds in agricultural crops, mainly corn (LeBaron et al., 2008; EPA, 2012). Human exposure to atrazine can occur through several routes. Agricultural workers and those living near farms can be exposed to atrazine dispersed in air through spraying (ATSDR, 2003). Exposure can also occur through contact with contaminated soils or ingestion of contaminated agricultural products (ATSDR, 2003). Water run-off from crops and lawn applications can get into ground and surface waters and contaminate

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drinking water wells (Munger et al., 1997). In water, atrazine breaks down to the following primary metabolites: desethyl atrazine (DEA), desisopropyl atrazine (DIA) and diaminochlorotriazine (DACT) (EPA, 2012).

In areas where atrazine is used, the general population can be exposed to atrazine analytes (i.e., atrazine parent compound or any of its metabolites) through contaminated drinking water (Munger et al., 1997; Ochoa-Acuna et al., 2009; Villanueva et al., 2005). Developing fetuses and children can be exposed in utero or through breast milk (Balduini et al., 2003; Whyatt et al., 2003). Atrazine is broken down rapidly in the body and eliminated primarily in urine, within 24–48 h (Catenacci et al., 1993). Although it does not bioaccumulate appreciably in humans, atrazine and its related compounds can persist in ground water, contaminating surface and drinking water sources (ATSDR, 2003). This persistent contamination of drinking water can result in continuous exposure in humans, and therefore can be of concern (UNEP and WHO, 2012).

In Great Britain, atrazine has mainly been used on maize and sweet corn crops (Fera Science Ltd, 2016). During the years 1990–1992, about 42,000 kg of atrazine was applied annually in Great Britain, with about 17,000 kg applied to about 10,000 ha annually in the South Western region of England (Fera Science Ltd, 2016). The European Union (E.U.) banned the use of atrazine in 2003 because of its persistent contamination of drinking and ground water above the E.U. recommended limit of 0.1 parts per billion (ppb) (Sass and Colangelo, 2006). However, several countries still use this herbicide including the US where it is a restricted use pesticide (ATSDR, 2003).

Animal studies have associated exposure to atrazine with hormone-related tumors and with alterations in reproductive functions suggesting potential endocrine disrupting effects from these exposures. In rats, postnatal exposure to atrazine induced an earlier onset (Cooper et al., 2007; Wetzel et al., 1994) and increased incidence of mammary gland tumors by disrupting the ovarian function (Cooper et al., 2007). Atrazine postnatal exposure also caused a disruption in estrus cycle in rats by impairing the ovulatory surge of luteinizing hormone (Wetzel et al., 1994). Lactational exposure to atrazine or DACT (Laws et al., 2000, 2003) and prenatal exposure to atrazine (Davis et al., 2011) in female rats resulted in delayed puberty (vaginal opening) in the offspring. Further, DACT was demonstrated to be as potent as atrazine in delaying puberty (Laws et al., 2003). However, the doses associated with delayed puberty in these studies were high (50,000–200,000 ppb daily).

Several studies have reported a secular trend towards earlier onset of puberty among girls in Europe and the United States (Aksglaede et al., 2008; Euling et al., 2008; McDowell et al., 2007; Parent et al., 2003; Semiz et al., 2008) with some reporting earlier age at onset of menarche (McDowell et al., 2007; Semiz et al., 2008). Early puberty is a risk factor for childhood risky behaviors (e.g., smoking, alcohol consumption and drug abuse) and adult-onset diseases (e.g., breast and ovarian cancer) (Gail et al., 1989; Moorman et al., 2009; vanJaarsveld et al., 2007; Walvoord, 2010). Epidemiologic studies of other endocrine disrupting chemicals (EDCs) suggest that in utero exposure to the estrogenic effect of EDCs is associated with early onset of puberty (Blanck et al., 2000; Vasiliu et al., 2004). Studies have also associated prepubertal exposure to EDCs with later onset of puberty (Windham et al., 2015; Wolff et al., 2015). Evidence from experimental studies suggests that atrazine analytes alter the timing of puberty in laboratory animals, however, such associations have not been investigated in humans. This study uses a nested case-control study design to measure atrazine analytes in maternal gestational urine as a proxy for in utero exposure of cases and controls to atrazine analytes, and examines the association with early menarche, as a marker of early puberty in girls.

2. Methods

2.1. Study population

Pregnant women living in the Bristol area, in the south west of England, United Kingdom, with an expected date of delivery from 1st April, 1991 to 31st December, 1992, were recruited to participate in the Avon Longitudinal Study of Parents and Children (ALSPAC) (Fraser et al., 2013). A total of 14,775 live births were included in the ALSPAC cohort (Boyd et al., 2013). Details about recruitment and the study participants have already been published (Fraser et al., 2013; Boyd et al., 2013). Beginning at the age of 8, a puberty questionnaire titled "Growing and Changing" was mailed to the participants each year until age 17 (except age 12) to collect information about the onset and progression of puberty in enrolled girls and boys (Rubin et al., 2009). The number of singleton girls available for follow-up was 5756 (Christensen et al., 2011), and only 3938 returned at least one valid and complete questionnaire (Christensen et al., 2010). The onset of menarche information in the questionnaire included the age and date of first menstrual period. The parents, the girls, or both completed the questionnaires (Rubin et al., 2009). The median age at onset of menarche for the girls who returned at least one puberty questionnaire (n = 3938) was determined using parametric survival analysis, and reported as 12.87 years (95% confidence interval [95% CI]): 10.82, 12.91) (Christensen et al., 2010). The questionnaire is available on the study website at http://www.bristol.ac.uk/media-library/sites/alspac/migrated/documents/ques-cb16a-mum-and-daughter-at-8.pdf. The website also contains detailed descriptions of available data accessible through a fully searchable online data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

The selection of cases and controls for the nested case-control study has been previously described (Christensen et al., 2011). In brief, only the 3682 singleton girls who returned at least 2 valid and complete "Growing and Changing" questionnaires from ages 8–13 years were considered for the study. From this number 218 were identified as having menarche before 11.5 years of age, and had one maternal gestational serum sample available for analysis. A subset of 174 cases also had one maternal gestational urine sample available for analysis. A random sample of 394 girls that had menarche at or after 11.5 years of age were selected as controls; 230 of these had one maternal gestational serum sample and 195 also had one maternal gestational urine sample available for analysis. We included only the 174 cases and 195 controls with analyzable maternal gestational urine samples in this study. The urine samples were collected from mothers as part of routine antenatal care at random times during pregnancy (between 1st April 1991 and 31st December 1992), (Fraser et al., 2013; Boyd et al., 2013) therefore, stage of gestation and timing of urine collection may vary among mothers' urine samples. Each urine sample was divided into small aliquots to maximize efficiency of the sample use, and banked at the University of Bristol (Boyd et al., 2013). The samples were stored at +4 or −20 °C 0–6 days after initial collection then stored between −10 and −20 °C until analysis. Only one aliquot per mother was used for this study. The ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board assessed and approved human subject protection (Christensen et al., 2011).

2.2. Exposure assessment

Banked urine samples from the University of Bristol were transferred to CDC's Division of Laboratory Sciences within the National Center for Environmental Health in 2008, and analyzed during the same year. The following atrazine analytes were measured using on-line solid phase extraction-isotope dilution-high performance liquid chromatography-tandem mass spectrometry (Panuwet et al., 2008, 2010): Atrazine, DACT, DEA, DIA, atrazine mercapturate, desethyl atrazine