Serum Dioxin Concentration and Age at Menarche

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a widespread environmental contaminant and known endocrine disruptor, is associated with a delay in onset of puberty in animal studies. *In utero* and lactational TCDD-exposure in rodents has been associated with delays in pubertal development (e.g., delayed vaginal opening, altered vaginal estrous cyclicity) [1, 2] and effects on ovarian function [1, 3], even at doses below those which induce overt maternal toxicity. A similar spectrum of reproductive alterations has been associated in rodents exposed *in utero* to other dioxin-like compounds including PCDDs, PCDFs, and PCBs [4-7].

To date, no epidemiologic studies have examined the association of TCDD exposure and age at menarche. Three studies, however, have examined the relation of dioxin-like compounds to pubertal development, with inconsistent conclusions. A study of daughters of Michigan women who had consumed polybrominated biphenyls (PBB) in food in 1973, found an earlier age at menarche among daughters whose mothers had higher serum PBB levels [8]. No differences were found in age at menarche of Taiwanese women who were exposed postnatally (but premenarche) to PCBs and PCDFs via consumption of contaminated rice oil (Yu-Cheng) compared to unexposed [9]. In Flemish adolescents, there was no relation of age at menarche with current serum levels of dioxin-like compounds as measured by Chemical Activated LUciferase gene eXpression bioassay toxic equivalents (CALUX-TEQ) or individual PCB congeners 118, 153, and 180 [10].

On July 10, 1976, as a result of a chemical explosion, residents of Seveso, Italy experienced the highest levels of TCDD exposure in a human population. Twenty years later (1996-1998), the Seveso Women's Health Study (SWHS), a retrospective cohort study, was initiated to determine whether the women were at higher risk for reproductive disease.

Among participants in SWHS, we have observed that TCDD levels are associated with an increase in menstrual cycle length among those who were premenarcheal at exposure, but not in those who were postmenarcheal at exposure [11]. Consistent with animal studies [12], this suggests that females may be particularly susceptible to the effects of TCDD during early stages of development,

e.g. *in utero* or pre-pubertal. Thus, here we examine the association of individual serum TCDD and age of menarche among women who were premenarcheal in 1976, at the time of explosion.

Material and Methods

SWHS is the first comprehensive epidemiologic study of the reproductive health of a female population exposed to TCDD. Women eligible for SWHS were one month to 40 years old in 1976, had resided in one of the most highly contaminated zones, A or B, and had adequate stored sera collected soon after the explosion [13]. Recruitment took place between March 1996 and July 1998. Of 1271 eligible women, 17 could not be contacted, and 33 had died or were too ill to participate. Of the 1221 women contacted, 981 (80%) agreed to participate. We limited this analysis to all 282 women who were premenarcheal on July 10, 1976, the date of the explosion.

Details of the study are presented elsewhere [13]. The data analyzed for the present analysis were based on information acquired during a detailed interview by a trained nurse-interviewer, who was blinded to the woman's serum TCDD level and Zone of residence. The interview gathered information on sociodemographic characteristics, personal habits, work history, and detailed gynecologic, menstrual, pregnancy and other medical history. Age at menarche was determined from the question, "At what age did you get your first menstrual period?"

For each participant, we selected the first serum sample collected between 1976 and 1981 that was of adequate volume (>0.5mL) for analysis. The TCDD concentration in these samples was measured by high-resolution mass spectrometry methods at the United States Centers for Disease Control and Prevention (Patterson et al. 1987). Values were reported on a lipid-weight basis in parts per trillion (ppt) (Aikin et al., 1987).

For the 282 women in this analysis, we measured TCDD in sera collected between 1976 and 1977 for 257 women; between 1978 and 1981 for 23 women; and in 1996 for two women whose earlier samples had insufficient volume. For women with detectable post-1977 TCDD measurements (n=20), the TCDD exposure level was back-extrapolated to 1976 using the Filser Model [14]. For non-detectable values (n=22), a serum TCDD level equal to one-half the detection limit was assigned [15].

Results and Discussion

On July 10, 1976, the average age of the 282 premenarcheal women was 6.9 years (SD = 3.7), and 158 (56 %) of them were less than eight years old. The average age at menarche reported by the 282 women was 12.8 years (SD = 1.6). The median serum TCDD level was 140.3 ppt (range: 3.6 - 56,000 ppt) for all premenarcheal women and 205.0 ppt (range = 3.6 – 56,000 ppt) for those who were less than eight years at exposure. Serum TCDD levels did not vary by reported age of menarche for either group ($p > 0.5$, ANOVA).

Among the 282 women who were premenarcheal at explosion, the adjusted Hazard Ratio (HR) associated with a 10-fold increase in TCDD was 0.95 (95 percent confidence interval: 0.83, 1.09); and the p-value for trend was $p = 0.46$. That is, there was no change in risk of onset of menarche with a 10-fold increase in TCDD (e.g., 10 to 100 ppt). When TCDD was categorized, there was also no evidence of a dose–response trend ($p = 0.65$).

In summary, individual serum TCDD measurements are not significantly related to age of menarche among women in the SWHS cohort. The women in this study experienced significant TCDD exposure during the postnatal but pre-pubertal developmental period. Animal evidence suggests *in utero* exposure may be the more sensitive route for the developing fetus, thus, continued follow-up of the offspring of the SWHS cohort is important.

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