

## Serum Dioxin Concentrations and Age at Menarche

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a widespread environmental contaminant, is associated with delays in pubertal development in animal studies. On 10 July 1976, as a result of a chemical explosion, residents of Seveso, Italy, experienced the highest levels of TCDD exposure experienced by a human population. Twenty years later, we initiated the Seveso Women's Health Study (SWHS), a retrospective cohort study of female residents of the most contaminated areas, to determine whether the women were at higher risk for reproductive disease. We examined the association of TCDD serum levels, based on measurements in serum collected soon after the explosion, with reported age at menarche among the 282 SWHS women who were premenarcheal at the time of the explosion. We found no change in risk of onset of menarche with a 10-fold increase in TCDD (e.g., 10–100 ppt; hazard ratio = 0.95; 95% confidence interval, 0.83–1.09; *p*-value for trend = 0.46). When TCDD levels were categorized, there was also no evidence of a dose–response trend (*p* = 0.65). In summary, we found that individual serum TCDD measurements are not significantly related to age at menarche among women in the SWHS cohort. The women in this study experienced substantial TCDD exposure during the postnatal but prepubertal developmental period. Given that animal evidence suggests *in utero* exposure has the most significant effect on onset of puberty, continued follow-up of the offspring of the SWHS cohort is important. **Key words:** dioxin, endocrine disruptors, environmental exposures, epidemiology, menarche, puberty, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 112:1289–1292 (2004). doi:10.1289/ehp.7004 available via <http://dx.doi.org/> [Online 10 June 2004]

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) constitute a group of polyhalogenated aromatic hydrocarbons that are persistent, widespread environmental contaminants, frequently detected at parts-per-trillion levels (lipid basis) in animals and humans throughout the industrialized world (Zook and Rappe 1994). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) is the most toxic congener within this group of compounds and has been shown to cause a wide variety of effects in animals, including altered reproductive development [Birnbaum 1994, 1995; International Agency for Research on Cancer (IARC) 1997]. Increasing evidence suggests that exposure to TCDD during earlier stages of development is particularly hazardous to reproductive development (Chaffin et al. 1996). *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 (Gray and Ostby 1995; Wolf et al. 1999)] and effects on ovarian function (Gray and Ostby 1995; Heimler et al. 1998), even at doses below those that 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 (Faqi

et al. 1998; Hamm et al. 2003; Muto et al. 2003; Sager and Girard 1994).

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 polychlorinated biphenyls (PCBs) in food in 1973 found earlier menarche in daughters whose mothers had higher serum PBB levels (Blanck et al. 2000). There were no differences in age at menarche in Taiwanese women who were exposed postnatally (but premenarche) to PCBs and PCDFs via consumption of contaminated rice oil (Yucheng) compared with unexposed women (Guo and Kao 2003). In Flemish adolescents, although breast development was inversely related, there was no relation of age at menarche to 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 (Den Hond et al. 2002).

On 10 July 1976, as a result of a chemical explosion, residents of Seveso, Italy, experienced the highest levels of TCDD exposure in a human population. Shortly after the explosion, a cohort of residents was established and exposure status was classified by

zone of residence (A, B, R, non-ABR) as determined by surface soil TCDD measurements (di Domenico et al. 1980). Twenty years after the explosion, we initiated the Seveso Women's Health Study (SWHS) to measure TCDD in previously stored blood samples and to assess associations of serum levels of TCDD with reproductive disease.

In the SWHS, we have observed that serum TCDD levels were associated with an increase in menstrual cycle length among those who were premenarcheal at exposure, but not in those who were postmenarcheal at exposure (Eskenazi et al. 2002). Consistent with animal studies (Chaffin et al. 1996), this suggests that females may be particularly susceptible to the effects of TCDD during early stages of development, for example, *in utero* or prepubertal exposure. In this article we report the results of the association of individual serum TCDD and age of menarche among women who were premenarcheal in 1976, at the time of the explosion.

### Materials and Methods

**Study population.** Women eligible for the SWHS were 1 month to 40 years of age 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. Enrollment began in March 1996 and ended in July 1998. Of 1,271 eligible women, 17 could not be located or contacted, 33 had died or were too ill to participate, and 240 declined to participate, leaving 981 women. The age distribution of those

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