Serum dioxin levels and age at menopause in women of Seveso

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Introduction

2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD or dioxin), a halogenated biphenyl that binds and activates the Ah receptor, is a contaminant of numerous industrial processes. Dioxin, a well-known human carcinogen, is also thought to disturb the development of the reproductive system. Laboratory studies have shown that dioxin and dioxin-like compounds, organochlorine pesticides including DDT and DDE, and some PCBs and PBBs are endocrine disrupters. Studies in rats and monkeys suggest that TCDD may affect ovarian function, both directly such as by the inhibition of estradiol synthesis or indirectly via the pituitary[1-3]. *In utero* and lactational TCDD exposure in rats has been associated with reduced ovarian weight as well as number of corpus lutea and pre-antral and antral follicles[4]. Postnatal TCDD exposure in rats has been associated with reduced ovarian weight gain, ovulation rate, and number of follicles as well as inhibition of follicular rupture[5], morphologic changes in the ovary and altered cyclicity with disruption of the estrous cycle[2, 5-8]. The observed reduction in follicle number suggests that TCDD may reduce follicular reserve and thus, result in earlier reproductive senescence[9, 10].

Menopause, the cessation of menstruation, is thought to be due to a loss of primordial ovarian follicles, resulting in the decline in estradiol production and the concomitant increase in circulating concentrations of follicle stimulating hormone. The age of menopause is believed to reflect the increased rate of atrophy of the ovarian follicles. Age at menopause has important health implications since women with early menopause are at higher risk for osteoporosis, cardiovascular disease and reproductive cancers[11, 12].

Chemicals that deplete or are toxic to ovarian follicles or to the hypothalamic-pituitary axis, e.g., by altering pituitary hormones or releasing factors, could result

in premature menopause. Thus far, few chemicals have been examined in relation to age of menopause, but consistent associations of smoking and earlier age of menopause provides evidence for the role of chemical toxicity in the timing of menopause in humans[13, 14]. However, there is only limited evidence to further suggest that endocrine disrupting chemicals impact the natural timing of menopause. In a recent analysis of breast cancer cases and controls (n= 1407 women), women living in North Carolina with DDE levels in the upper tenth percentile had an earlier onset of natural menopause than women with levels below the median, but PCB levels were unrelated[15]. Similarly, compared to unexposed controls, women from the Yucheng cohort who had been poisoned postnatally by cooking oil contaminated with PCBs had no difference in the percentage of women who experienced menopause, and no difference in the mean age at menopause[16]. In regards to TCDD, amenorrhea was observed in an Austrian woman who had been TCDD poisoned and had extremely high levels of TCDD in serum (144,000 ppt)[17]. Additional evidence for the potential effects of TCDD on the menstrual cycle derives from our earlier findings[18], showing an elongation of the menstrual cycle, but only in women who were premenarcheal at the time of exposure.

In the present investigation, we examine the relationship of TCDD and age of onset of menopause in a population of women residing near Seveso, Italy in 1976, at the time of a chemical plant explosion. These women, who participated in the Seveso Women's Health Study, were exposed to the highest levels of TCDD known in residential populations[19]. Serum specimens collected near the time of the explosion allowed for individual estimates of exposure.

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[20]. Recruitment began in March 1996 and was completed in 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. For this analysis, we included the 616 women who were at least 35 years old at the time of interview but had not reached natural (n=3) or surgical (n=4) menopause prior to July 10, 1976, the date of the explosion.

Details of the study are presented elsewhere[20]. Briefly, participation included informed consent, venipuncture, personal interview, gynecologic examination and transvaginal ultrasound, medical records, and 3-month daily menstrual diary. For the interview, each woman was interviewed by a highly trained nurse-interviewer who was blind to TCDD level and residence of the woman. Information collected during the interview included sociodemographic information, personal habits, work history, detailed gynecologic and other medical history, detailed pregnancy history including time to conception of first pregnancy after the accident, menstrual cycle history and exposure to TCDD.

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[21]. Values were reported on a lipid-weight basis in parts per trillion (ppt)[22]. For the 616 women in this analysis, we measured TCDD in sera collected between 1976 and 1977 for 564 women; between 1978 and 1982 for 28 women; and between 1996 and 1997 for 24 women whose earlier samples had insufficient volume. For women with post-1977 TCDD measurements (n=52), the TCDD exposure level was back-extrapolated to 1976 using the first–order kinetic model[23]. For non-detectable values (n=22), a serum TCDD level equal to one-half the detection limit was assigned[24].

We defined age at menopause based on the standard WHO definition[25], 12 or more months of amenorrhea not due to other obvious causes such as pregnancy, lactation, and medical conditions. The 616 women were classified into the following menopause categories: “premenopause” (n=260); “natural menopause (n=169); “surgical menopause” (n=83) if the woman had a hysterectomy and/or unilateral or bilateral oophorectomy; “impending menopause” (n=13) if the woman had menstruated within 12 months, but not in the two months prior to interview or exam, and if her amenorrhea could not be explained by another condition; “perimenopause” (n=33) if the woman reported her cycles had become less predictable (irregular or longer) in the previous 2 to 5 years; and “other” (n=58) if the woman’s menopausal status could not be determined due to reasons including current pregnancy, current lactation, current oral contraceptive or other hormone use, or past chemotherapy treatment.

We examined the relation of serum TCDD and age of onset of menopause using Cox proportional hazards modeling. We considered serum TCDD level as a continuous (log base 10) and a categorical variable based on quintiles of exposure. Women classified as “natural menopause” failed at their age (in years) at last menstrual period. Women classified as “surgical menopause” were censored at their age at which they had surgery. “Other” women were censored at the age at which they became other (e.g., age they began oral contraceptives). “Premenopause”, “perimenopause”, and “impending menopause” women were censored at their age at interview.

Results and Discussion

The average age at interview of the 616 women was 47.8 (SD= 8.1) years, and ranged from 35 to 63 years. Overall, the median TCDD exposure level was 43.7 ppt, lipid-adjusted, and ranged from 2.5 to 6,320 ppt. Of the 169 (27%) women classified as "natural menopause", the average age at menopause was 49.2 (S.D.= 3.7) years. The median serum TCDD levels by menopause category are presented in Table 1.

Table 1. Distribution of serum TCDD levels by menopause category among women, 35–63 years, Seveso Women's Health Study, Italy, 1996-1998

Category	n (%)	TCDD median (IQR)
Natural menopause	169 (27)	45.8 (28 - 100)
Surgical menopause	83 (14)	43.4 (28 - 98)
Impending menopause	13 (2)	43.8 (24 - 105)
Perimenopause	33 (5)	36.5 (22 - 85)
Premenopause	260 (42)	43.6 (21 - 91)
Other	58 (9)	39.6 (17 - 85)
Total	616 (100)	43.7 (24 - 95)

In univariate analysis, the hazard ratio (HR) associated with a 10-fold increase in serum TCDD level (e.g., 10 to 100 ppt) was 1.02 (95 percent confidence interval: 0.78, 1.33); and the p-value for trend was $p = 0.89$. As presented in Table 2, when TCDD was categorized into 5 groups with equal numbers of women, the risk of earlier onset of menopause appears to trend upward until the highest exposure group.

Table 2. Unadjusted hazard ratio (95% confidence interval) for age at natural menopause by TCDD quintile for women, 35 – 63 years, Seveso Women's Health Study, Italy, 1996-1998

Serum TCDD (ppt, lipid-adjusted)	n (%)	Hazard Ratio (95% CI)
< 20.4	123 (20)	1.00
20.4 - 34.2	123 (20)	1.08 (0.66 – 1.76)
34.3 - 54.1	123 (20)	1.42 (0.89 – 2.27)
54.2 - 118.0	124 (20)	1.63 (0.97 – 2.73)
> 118.0	123 (20)	1.03 (0.60 – 1.78)

In summary, in univariate analysis, we find little evidence of a clear linear dose-response relationship of serum TCDD levels and earlier onset of menopause. Instead, we observe a linear

trend of earlier age of menopause for women with serum TCDD levels up to 118 ppt, but not higher. Results of the multivariate analysis, adjusting for potential confounding factors, will be presented.

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References

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