Serum Dioxin Concentrations and Menstrual Cycle Characteristics

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Received for publication August 29, 2001; accepted for publication April 24, 2002.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a widespread industrial environmental contaminant. Animal studies suggest that TCDD exposure alters the estrus cycle. Twenty years after a 1976 industrial explosion in Seveso, Italy, the authors interviewed female residents to determine whether there was an association between TCDD exposure and current menstrual cycle characteristics. The authors analyzed serum samples collected soon after the explosion to quantify individual TCDD levels. Among women who were premenarcheal at the time of the explosion, a 10-fold increase in serum TCDD level was associated with a lengthening of the menstrual cycle by 0.93 days (95% confidence interval (CI): –0.01, 1.86) and a reduction in the odds of scanty menstrual flow (adjusted odds ratio = 0.33, 95% CI: 0.10, 1.06). However, among women who were postmenarcheal at the time of the explosion, TCDD was not associated with menstrual cycle length (adjusted β = –0.03 days, 95% CI: –0.61, 0.54) or scantiness of flow (adjusted odds ratio = 1.36, 95% CI: 0.70, 2.64). In both menarche groups, TCDD levels were associated with decreased odds of having irregular cycles (adjusted odds ratio = 0.46, 95% CI: 0.23, 0.95) but were not related to days of flow (adjusted β = 0.16 days, 95% CI: –0.08, 0.41). These results are consistent with effects of TCDD on ovarian function noted in some animal species and with greater sensitivity to TCDD during development. Am J Epidemiol 2002;156:383–92.

Abbreviations: CI, confidence interval; PCB(s), polychlorinated biphenyl(s); SD, standard deviation; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

The compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or dioxin, is the most toxic member of a class of planar halogenated aromatic hydrocarbons (1). It is a widespread environmental contaminant produced by various industrial chemical reactions and combustion processes, including waste incineration. It is extremely stable and water-insoluble, with a long half-life in humans, ranging from 7 years to 9 years (2).

Findings from a number of animal studies have suggested that exposure to TCDD may impact the estrus cycle. Studies in rats and monkeys have suggested that TCDD may affect ovarian function, either directly and/or indirectly via the pituitary gland (3–6). Dose-related reductions in ovulation rate, reduced numbers of follicles, morphologic changes in the ovary and uterus, alterations in steroid levels, and altered cyclicity have been reported in rodents fed high doses of TCDD (3, 4, 7–12). Anovulatory patterns of decreased estradiol and progesterone levels were found in adult rhesus...
monkeys fed 500 ppt TCDD in the diet for 9 months; however, no changes in the length, intensity, or duration of the menstrual cycle were noted (13, 14). Long-term adverse effects on ovarian function after acute exposure to TCDD have been reported in adult cynomolgus macaques (6). One year after a single oral dose of TCDD (1, 2, or 4 µg/kg body weight), three of four monkeys in the high-dose group still had no evidence of menstrual cycles, with decreased ovarian steroidogenesis (6). However, in a study of adult cynomolgus monkeys exposed to TCDD daily at low doses (0, 1, 5, or 25 ng/kg body weight) for 1 year, no changes in menstrual cycle length, bleeding duration, or ovulation based on hormone levels were reported (15). A recent case report of secondary amenorrhea with decreased estradiol and progesterone levels in an Austrian woman with TCDD exposure of 144,000 ppt (~25 µg/kg body weight) suggests that, at least at very high doses, TCDD can affect menstruation in humans (16).

Although, to our knowledge, no studies have been conducted to examine the potential effects of TCDD exposure on the human menstrual cycle, there have been three studies of women exposed to other halogenated aromatic compounds (17–19). Taiwanese women aged 15–35 years who ingested fish oil contaminated with polychlorinated biphenyls (PCBs) and polychlorinated dibenzo furans were twice as likely as nonexposed women to report abnormally light menstrual bleeding 15 years later. There was no difference in their reports of heavy menstrual periods or irregular menstrual cycles. There was no evidence of a dose-response relation in the subsample of women with measured serum PCB levels (17). In the second study, women who ate PCB-contaminated sport fish from Lake Ontario more than once per month in 1991 or for more than 7 years or whose total intake was ranked as moderate-to-high (based on years of consumption, frequency, and amount consumed) had significant reductions in menstrual cycle length ranging from a half day to 1 day (18). In the third study, 58 percent of 81 Japanese women reported irregular menstrual cycles 2 years after consuming PCB-contaminated rice oil (“yusho”). Urinary excretion of estrogens, pregnanediol, and pregnanetriol tended to be low in this group (19).

In 1976, an explosion at a trichlorophenol-manufacturing plant near Seveso, Italy, resulted in the highest known TCDD exposure in human residential populations (20, 21). Twenty years later, we conducted a historical cohort study, the Seveso Women’s Health Study, to determine whether there was an association between TCDD exposure and women’s reproductive health, including menstrual cycle characteristics. Serum samples collected soon after the explosion rendered it possible to quantitatively individual TCDD exposures (22). Given that exposure levels for some of the women in Seveso exceeded levels that have produced ovarian dysfunction in animals (4, 8, 23, 24), we thought it likely that if humans are as sensitive as animals to the effects of TCDD, we would see menstrual cycle changes in this cohort. Because studies in a variety of species have suggested that developing organ systems are more susceptible to TCDD than the organs of animals that have reached maturity (25), we hypothesized that any effects of TCDD exposure were more likely to appear among women who had been exposed prior to menarche.

MATERIALS AND METHODS

Study population

On July 10, 1976, an explosion occurred in the ICMESA chemical factory near Seveso, Italy, approximately 25 km north of Milan. Possibly up to 30 kg of TCDD were deposited over an 18-km² area (26). The area was subsequently divided into exposure zones based on soil TCDD levels. Zone A was the most heavily contaminated area, and zone B was the next most heavily contaminated (27). As part of a health assessment, blood samples were collected from Seveso residents soon after the explosion for clinical chemical testing; the remaining portion of each serum sample was stored for future studies (20).

The follow-up study of the women was conducted in 1996–1997, approximately 20 years after the explosion. Women eligible for inclusion in the Seveso Women’s Health Study were those who were aged 40 years or younger in 1976, had adequate amounts of stored serum collected between 1976 and 1980, and had resided in zone A or zone B at the time of the accident. A total of 1,271 women met these criteria. Seventeen women could not be located or reached, and 33 had died or were too ill to participate. Of the 1,221 women contacted, 981 (80 percent) agreed to participate. For the current analysis of menstrual function, we excluded women over 44 years of age (n = 382) to avoid confusion of perimenopausal events with menstrual abnormalities. We also excluded women with surgical or natural menopause (defined as not having menstruated for the last 12 months) (n = 7), women with Turner’s syndrome (n = 3), and women who, in the past year, had been pregnant (n = 45), had breastfed (n = 10), or had used an intrauterine device (n = 35) or a hormonal medication such as oral contraceptives (n = 198). Thus, the final sample included 301 women. Informed consent was obtained from all women prior to participation.

Procedure

The data analyzed for the present study were based on information acquired during a detailed interview conducted by a trained nurse-interviewer who was blinded to the woman’s serum TCDD level and zone of residence. The interview covered sociodemographic data, personal habits, work history, and detailed information on reproductive history (gynecologic, menstrual, and pregnancy-related factors) and other medical history.

We derived information on the menstrual cycle from responses to the following questions: 1) “In the past year, on average, how long have your cycles been?” 2) “In the past year, were your cycles regular (that is, the number of days from the start of one period to the start of the next was about the same, give or take 4 days)?” 3) “In the past year, on average, how many days did your flow last?” 4) “During the past year, how would you describe the heaviness of your menstrual flow—scanty, moderate, or heavy?” In addition,
we obtained and abstracted data from medical records for all obstetric and gynecologic conditions. Additional details on the study methods have been provided elsewhere (23).

**Laboratory analyses**

We preferentially selected the earliest serum sample that had been collected between July 1976 and 1985 and stored at −20°C. If the sample volume was inadequate (<0.5 ml) and if another specimen had been collected within 1 year of the first, we pooled the two samples. Lipid levels, including levels of total cholesterol and triglycerides (28), and sodium concentrations were measured for each serum sample to ensure that it was not desiccated. Archived sera were sent on dry ice to the US Centers for Disease Control and Prevention (Atlanta, Georgia) for TCDD analysis by high-resolution mass spectrometry (29). Values were reported on a lipid-weight basis in parts per trillion (ppt) (30).

For 29 women, only a post-1977 serum sample had adequate volume for TCDD analysis. If TCDD levels measured in these post-1977 samples were greater than 10 ppt (n = 26), the TCDD exposure level was back- extrapolated to 1976 using the Filser model (31) for women aged 16 years or younger in 1976 (n = 20) and the first-order kinetic model (2) otherwise (n = 6). For nondetectable values (n = 26), a value of one half of the detection limit was assigned (32).

**Statistical analysis**

Statistical analyses were performed using Stata software, version 7.0 (33). Serum TCDD level was logarithmically transformed (base 10) and considered as a continuous variable. The menstrual cycle outcomes assessed included length of the menstrual cycle in days (continuous); number of days of menstrual flow (continuous); regularity of menstrual cycles (irregular (length varied by more than 4 days) vs. regular); and heaviness of menstrual flow (characterized as scanty, heavy, or moderate). Women who reported having irregular cycles (n = 24) were excluded from the analysis of menstrual cycle length. We examined the relations of TCDD level to irregular cycle length and heaviness of menstrual flow using logistic regression. For heaviness of flow, we compared scanty flow with moderate or moderate/heavy flow and heavy flow with moderate or moderate/scanty flow in separate regression models. We examined the relation of TCDD to cycle length and days of flow using multiple linear regression. For all regression analyses, we report nonparametric (Huber, sandwich) standard errors, which are valid even when conventional assumptions for regression analyses are violated (34).

We examined numerous potential confounders and effect modifiers reported in the literature (35), including age at interview, education, parity, smoking, body mass index (weight (kg)/height (m)²), alcohol and coffee consumption, age at menarche, sexual activity, number of hours worked per week, number of hours of physical exercise per week, chronic illness, and abdominal surgeries. Age and body mass index were modeled as both continuous variables and categorical variables; all other variables included in the final models were modeled as categorical variables (table 1). We hypothesized that any effect of TCDD exposure would be stronger in women who had been premenarcheal at the time of the explosion, and we tested this hypothesis by including an interaction term for TCDD and menarcheal status. Therefore, separate slopes for log₁₀ TCDD were estimated for women who were premenarcheal and women who were postmenarcheal at the time of the explosion. Covariates that confounded the relation between TCDD level and the outcome in bivariate analyses (defined as a >10 percent change in the coefficient for TCDD) were entered into a multivariate model and tested by likelihood ratio test. In addition, we kept age at menarche in the final model, because there is some evidence in the literature that it is related to subsequent menstrual cycle characteristics (36, 37).

To ensure that women who might have been perimenopausal were not influencing the results, we reran the final analyses after excluding women older than age 40 years.

**RESULTS**

Table 1 presents data on menstrual cycle characteristics and TCDD levels according to the women’s sociodemographic characteristics, reproductive histories, and personal habits. On average, the women were 33.3 years old (standard deviation (SD), 6.8; range, 20–44) at interview. All were Caucasian, most had completed only high school, 15 percent had not yet initiated sexual activity, approximately half were parous and had breastfed, 17 percent were overweight (body mass index, >26), 24 percent were current smokers, and 11 percent exercised for 3 or more hours per week. The average age at menarche was 12.5 years (SD, 1.4); 45 percent of the women had been premenarcheal at the time of the explosion. The average cycle length was 28.2 days (SD, 2.7), with an average of 5.0 days of flow (SD, 1.3). Eight percent (n = 24) of the women reported having irregular cycles, 10 percent (n = 30) reported having scanty flow, and 28 percent (n = 84) reported having heavy flow. The average length of the menstrual cycle decreased with age, and cycles were shorter among women who had initiated sexual activity, women who had breastfed, and women who were currently getting little or no exercise. Women with scanty flow were more likely to be older.

On average, the women were aged 12.9 years (SD, 6.7; range, 0–24) at the time of the explosion. Women who had been premenarcheal at the time of the explosion (n = 134) were, on average, aged 6.7 years (SD, 3.7; range, 0–17) at the time of the explosion and aged 27.1 years (SD, 3.8; range, 20–37) at interview. Women who had been postmenarcheal at the time of the explosion (n = 167) were, on average, aged 17.9 years (SD, 3.9; range, 11–24) at the time of the explosion and aged 38.3 years (SD, 3.9; range, 31–44) at interview.

Log₁₀ serum TCDD level was positively associated with menstrual cycle length; that is, a 10-fold increase in TCDD level (e.g., from 10 ppt to 100 ppt) was associated with an increase of approximately 0.55 days (95 percent confidence interval (CI): −0.01, 1.10) in estimated cycle length. As table 2 shows, the strength of the relation decreased slightly after
adjustment for age, age at menarche, prior coitus, and exercise (adjusted $\beta = 0.40$ days, 95 percent CI: $-0.14$, $0.94$). When we stratified the data by menarcheal status at the time of the explosion, the association between increasing serum TCDD level and longer cycles was present only among women who had been premenarcheal at the time of the explosion. Figure 1 shows the unadjusted linear relation between menstrual cycle length and serum TCDD level by menarcheal status at time of explosion. For women who had been premenarcheal at the time of the explosion, a 10-fold increase in serum TCDD level was associated with an increase in menstrual cycle length of almost 1 day (adjusted $\beta = 0.93$ days, 95 percent CI: $-0.01$, $1.86$), but there was no association among women who were postmenarcheal (adjusted $\beta = -0.03$ days, 95 percent CI: $-0.61$, $0.54$) ($p$ for interaction = 0.08). When we excluded the two women with cycle lengths greater than 38 days (40 and 48 days), the results remained similar, though diminished (premenarcheal: adjusted $\beta = 0.79$ days, 95 percent CI: $-0.09$, $1.67$; postmenarcheal: adjusted $\beta = -0.01$ days, 95 percent CI: $-0.58$, $0.55$) ($p$ for interaction = 0.13). We also conducted the final analysis with inverse cycle length (1/cycle length) included in the model as the dependent variable; this would have served to downweight extreme values. The results showed a 0.72-day increase in cycle length for the premenarcheal group ($p = 0.10$) and no effect in the postmenarcheal group ($p$ for interaction = 0.14).

There was no relation between serum TCDD level and days of menstrual flow (adjusted $\beta = 0.16$ days) (table 2). No difference was observed between women who were premenarcheal at the time of the explosion and women who were postmenarcheal.

Table 3 presents adjusted odds ratios associated with TCDD level for heaviness of menstrual flow and irregular cycle length. The numbers of women reporting scanty or irregular flow were small. The adjusted odds ratio for heavy flow versus moderate flow was 0.95 (95 percent CI: $0.61$, $1.50$), suggesting that there was no difference between the two groups. Hence, we combined the moderate- and heavy-flow groups for comparison with the group with scanty flow.
Reported scanty flow was somewhat associated with serum TCDD level (unadjusted odds ratio = 0.60, 95 percent CI: 0.35, 1.03), but the association was diminished after data were adjusted for age at interview and age at menarche (adjusted odds ratio = 0.84, 95 percent CI: 0.44, 1.61). The results were similar when scanty flow was compared with moderate flow alone. However, when data were stratified by menarcheal status, women who had been premenarcheal at the time of the explosion had reduced odds of scanty flow (adjusted odds ratio = 0.33, 95 percent CI: 0.10, 1.06), while women who had been postmenarcheal did not (adjusted odds ratio = 1.36, 95 percent CI: 0.70, 2.64) \( p \) for interaction = 0.03.

Serum TCDD levels were associated with reduced odds of having an irregular cycle after adjustment for age at interview and age at menarche. The adjusted odds ratio associated with a 10-fold increase in serum TCDD level was 0.46 (95 percent CI: 0.23, 0.95). Associations were similar among women who had been premenarcheal and women who had been postmenarcheal at the time of the explosion.

When we restricted the analyses to women who were aged 40 years or less at the time of interview, the above results remained the same (data not shown).

**DISCUSSION**

Results from this study of women residing in Seveso, Italy, at the time of the ICMESA chemical explosion suggest that there is an association between serum TCDD levels and certain menstrual cycle characteristics and that this association varies by menarcheal status at exposure. Among women who were premenarcheal at the time of the explosion, higher serum TCDD levels were associated with longer menstrual cycles but reduced odds of scanty flow. However, among women who were postmenarcheal at the time of the explosion, higher TCDD levels were not associated with cycle

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**TABLE 1. Continued**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>TCDD level (ppt)</th>
<th>Mean length of menstrual cycle (days) (SD)</th>
<th>Irregular menstrual cycles</th>
<th>Mean no. of days of menstrual flow (SD)</th>
<th>Heaviness of menstrual flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Median IQR</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Breastfeeding</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Never</td>
<td>166</td>
<td>55</td>
<td>126.5* 39–288 28.4* (3.1)</td>
<td>10 6</td>
<td>4.9 (1.2)</td>
<td>14 8 110 66 42 25</td>
</tr>
<tr>
<td>Ever</td>
<td>135</td>
<td>45</td>
<td>48.8 25–85 27.8 (2.0)</td>
<td>14 10</td>
<td>5.0 (1.5)</td>
<td>16 12 77 57 42 31</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>&lt;19.8</td>
<td>74</td>
<td>25</td>
<td>120.1 51–309 28.1 (2.7)</td>
<td>5 7</td>
<td>5.1 (1.2)</td>
<td>5 7 49 66 20 27</td>
</tr>
<tr>
<td>19.8–26.0</td>
<td>175</td>
<td>58</td>
<td>63.0 30–180 28.2 (3.0)</td>
<td>17 10</td>
<td>4.9 (1.2)</td>
<td>17 10 111 63 47 27</td>
</tr>
<tr>
<td>26.1–29.0</td>
<td>31</td>
<td>10</td>
<td>59.4 17–222 28.1 (1.6)</td>
<td>1 3</td>
<td>5.5 (1.8)</td>
<td>4 13 17 17 17 32</td>
</tr>
<tr>
<td>&gt;29.0</td>
<td>21</td>
<td>7</td>
<td>30.7 16–107 28.2 (1.8)</td>
<td>1 5</td>
<td>4.6 (1.7)</td>
<td>4 19 10 48 7 33</td>
</tr>
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<td>Current smoking (cigarettes/day)</td>
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<td>0</td>
<td>228</td>
<td>76</td>
<td>72.4 32–209 28.1 (2.9)</td>
<td>16 7</td>
<td>5.0 (1.3)</td>
<td>21 9 144 63 63 28</td>
</tr>
<tr>
<td>1–10</td>
<td>47</td>
<td>16</td>
<td>61.6 22–169 28.3 (1.9)</td>
<td>4 9</td>
<td>4.9 (1.2)</td>
<td>4 9 29 62 14 30</td>
</tr>
<tr>
<td>&gt;10</td>
<td>26</td>
<td>8</td>
<td>45.4 29–131 28.0 (2.0)</td>
<td>4 15</td>
<td>4.8 (1.7)</td>
<td>5 19 14 54 7 27</td>
</tr>
<tr>
<td>Current exercise (hours/week)</td>
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<td></td>
<td></td>
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<tr>
<td>&lt;3</td>
<td>268</td>
<td>89</td>
<td>65.6 30–192 28.0* (2.3)</td>
<td>22 8</td>
<td>5.0 (1.3)</td>
<td>28 10 167 62 73 27</td>
</tr>
<tr>
<td>≥3</td>
<td>33</td>
<td>11</td>
<td>81.9 28–285 29.6 (4.5)</td>
<td>2 6</td>
<td>5.3 (1.3)</td>
<td>2 6 20 61 11 33</td>
</tr>
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<td>Previous abdominal surgery¶</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>87</td>
<td>29</td>
<td>44.4 27–69 27.9 (1.7)</td>
<td>9 10</td>
<td>5.2 (1.5)</td>
<td>13 15 51 59 23 26</td>
</tr>
<tr>
<td>No</td>
<td>214</td>
<td>71</td>
<td>99.1 34–285 28.3 (3.0)</td>
<td>15 7</td>
<td>4.9 (1.2)</td>
<td>17 8 136 64 61 29</td>
</tr>
</tbody>
</table>

* \( p \leq 0.05.
† TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; SD, standard deviation; IQR, interquartile range.
‡ Length of the menstrual cycle varied by more than 4 days.
§ Weight (kg)/height (m)².
¶ Includes surgery involving the uterus, ovaries, vagina, bowel, stomach, spleen, gallbladder, or kidney; does not include appendectomy; includes dilation and curettage, tubal ligation, cesarean section, and laparoscopy.
length or heaviness of flow. Among both pre- and post-menarcheal women, serum TCDD levels were inversely associated with the odds of having irregular cycles but were not associated with number of days of menstrual flow.

Our finding of longer cycles among women who were premenarcheal at exposure may have been partly driven by a few outliers. Nevertheless, this finding may be biologically plausible and is consistent with observations in animal studies of an association of TCDD exposure with ovarian dysfunction (3, 4, 7–10) and effects on steroidogenesis (4, 38, 39). TCDD may act as an endocrine disruptor in women exposed prior to menarche by altering the hypothalamic-pituitary-gonadal regulatory axis—for example, by lowering gonadotropin secretion or by altering the sensitivity of the ovarian follicles to gonadotropins. Such a disruption could result in an elongation of the follicular or secretory phase of the cycle or in a higher frequency of anovulatory cycles, both of which could manifest in longer cycles (40). The fact that we did not observe an association with serum TCDD in women exposed postmenarcheally suggests either that any perturbations due to exposure may have been compensated for during the approximately 20 years since the explosion or that once puberty has commenced, “normal” regulation of gonadotropins may be more resistant to perturbation (Bill Lasley, University of California at Davis, personal communication, 2002). The report by Treflar et al. (41) of smaller standard deviations for menstrual cycle length through the “middle life” of the menstrual experience (ages 20–40 years) provides further evidence that the menstrual cycles of older women may be more difficult to disrupt.

Our finding of reduced odds of scanty flow with no concomitant increase in heavy flow among women exposed prior to menarche is not entirely consistent with an increase in ovarian dysfunction. Although other studies of menstrual cycle characteristics have also collected data on the heaviness of women’s menstrual flow (35, 42–44), to our knowledge there has been little assessment of the reliability or validity of this measure. Ham et al. (45) reported that patients and their physicians independently agreed on the patient’s cycle length and on duration and scantiness of flow but not on the heaviness of flow. Hallberg et al. (46) found that mean blood loss was greater for women who reported heavy bleeding than for women who reported light or moderate bleeding. However, they found that 37 percent of the women with high blood loss (>80 ml) reported their bleeding as moderate, but a smaller fraction (14 percent) of women with low blood loss (<20 ml) reported their bleeding as heavy. Thus, scanty flow may be more reliably reported than heavy flow. In addition, given that the women were unaware of their serum TCDD level, there is no reason to believe that there was systematic bias in their reporting.

In this study, we also found a decrease in menstrual irregularity associated with TCDD exposure. Reported irregular cycle length, when defined in a similar fashion, has been validated against daily menstrual diary records (47). Nevertheless, because of the antiestrogenic effects of TCDD, we expected an increase in irregularity. In part, our observation may be an artifact of excluding the women who reported taking hormones to regulate their cycle. When we included those women (n = 29) in the analysis as having irregular cycles, serum TCDD level was no longer associated with irregular menstrual cycles (adjusted odds ratio = 0.67, 95 percent CI: 0.40, 1.10).

Our results are not entirely consistent with findings obtained in human cohorts exposed to PCBs. Mendola et al. (18) reported a shortening—not a lengthening—of the menstrual cycle in association with consumption of PCB-contaminated fish. However, their estimate of exposure was

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**TABLE 2. Adjusted beta coefficients from linear regression of menstrual cycle length and days of menstrual flow on log\(_2\) 2,3,7,8-tetrachlorodibenzo-p-dioxin level, overall and by menarcheal status at the time of the ICMESA chemical explosion, Seveso Women’s Health Study, Italy, 1996–1998**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total no.</th>
<th>All women</th>
<th>Menarcheal status at time of explosion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adjusted β* 95% confidence interval</td>
<td>Premenarcheal (n = 134)</td>
</tr>
<tr>
<td>Menstrual cycle length (days)</td>
<td>277</td>
<td>0.40† −0.14, 0.94</td>
<td>0.93† −0.01, 1.86</td>
</tr>
<tr>
<td>Days of menstrual flow</td>
<td>301</td>
<td>0.16§ −0.08, 0.41</td>
<td>0.18§ −0.15, 0.51</td>
</tr>
</tbody>
</table>

* Change in days per 10-fold increase in serum 2,3,7,8-tetrachlorodibenzo-p-dioxin level (e.g., from 10 ppt to 100 ppt).
† Adjusted for age at interview, age at menarche, prior coitus, and exercise.
‡ Difference in the relation of the menstrual cycle characteristic to 2,3,7,8-tetrachlorodibenzo-p-dioxin level between premenarcheal women and postmenarcheal women.
§ Adjusted for age at interview, age at menarche, and prior surgery (including cesarean section, laparoscopy, dilation and curettage, cerclage, hysteroscopy, cholecystectomy, tubal ligation, and splenectomy).
based on self-reported fish consumption and may not have accurately reflected body burden. In addition, the effects of PCBs may differ from those of TCDD, especially if the PCB congeners were not dioxin-like. Yu et al. (17) found that Taiwanese women exposed to PCB-contaminated cooking oil ("yucheng") who were postmenarcheal at exposure were more likely to have abnormally light menstrual periods, but women who were premenarcheal at exposure were not included. In addition, they found no difference between exposed and unexposed women in terms of the proportion reporting irregular cycles. However, compared with the current study, women in the yucheng study were older, with more than one quarter of the women being menopausal at the time of interview and half of the women being aged 40 years or older (17). Given that the women were asked about their typical cycles, many of them were required to recall events that had occurred many years in the past. In the study of Japanese women exposed to PCB-contaminated rice oil ("yusho"), over half reported irregular menstrual cycles 2 years after exposure (19). However, the relation of these outcomes to serum PCB levels was not reported.

Our study had several limitations. We could only assess the long-term effects of TCDD exposure. We asked women to report on their current cycles, i.e., their cycles over the previous year, not during the time immediately following the accident when their body burdens were highest. We did not measure TCDD in serum concurrently; instead we assumed that TCDD level at the time of the explosion was the relevant

**FIGURE 1.** Menstrual cycle length by serum 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) level among women who were premenarcheal (panel A) and women who were postmenarcheal (panel B) at the time of the ICMESA chemical explosion, Seveso Women's Health Study, Italy, 1996–1998.
measure, given the long half-life of TCDD and the fact that the follow-up period was the same for all women. We used retrospective reporting of cycle characteristics over the previous year, which has been shown in other studies to differ from data collected prospectively from daily diaries (48). In addition, we had relatively few women with negligible exposure or no exposure as based on current background levels (<5 ppt TCDD) (49), although the range of exposures was wide. Finally, we excluded a large number of women who were using intrauterine devices or oral contraceptives or other hormones (n = 233). As expected, these women were younger than the women who were not excluded. However, women using hormones or intrauterine devices had median TCDD levels similar to those of other women.

This study had several strengths. It was a population-based cohort study, and information was collected from highly trained nurse-interviewers. In addition, a large proportion of the 1976 cohort participated. We were able to confirm with medical records the information the women provided about their gynecologic conditions. Perhaps one of the greatest strengths of the study was our ability to quantify individual TCDD levels from serum collected soon after the accident. Furthermore, the exposure assessed was primarily exposure to TCDD; levels of other polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans were not elevated above background levels (20). Other exposed residential populations, such as those studied in Vietnam (Agent Orange) (50), Taiwan (contaminated rice oil) (51), Japan (contaminated rice oil) (52), and Russia (industrial contamination) (53), either had few or no biologic measurements of exposure or were exposed to a mixture of higher chlorinated dioxins, furans, and PCBs. Thus, to our knowledge, the Seveso Women’s Health Study was the first cohort study of TCDD in a population with known exposure levels. If TCDD alters the menstrual cycle, we were most likely to observe it in this large cohort of women with a wide range of exposures as assessed by individual TCDD measurements.

In conclusion, our results suggest that past TCDD exposure may be associated with small changes in current menstrual cycle characteristics, especially for women who were premenarcheal at the time of their exposure. These results are consistent with the endocrine-disrupting effects of TCDD noted in animal studies and with differing sensitivity by developmental stage at exposure.

ACKNOWLEDGMENTS

This study was supported by grants R01 ES07171 and F06 TW02075-01 from the US National Institutes of Health (Bethesda, Maryland), grant R82471 from the US Environmental Protection Agency (Washington, DC), grant EAM1977 from the Endometriosis Association (Milwaukee, Wisconsin), grant 2P30-ES001896-17 from the US National Institute of Environmental Health Sciences (Research Triangle Park, North Carolina), and grant 2896 from Regione Lombardia and Fondazione Lombardia Ambiente (Milan, Italy).

The authors are grateful to Stefania Casalini for coordinating data collection at the Hospital of Desio and to Wayman Turner and his staff for carrying out serum 2,3,7,8-

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**TABLE 3.** Unadjusted and age-adjusted odds ratios from logistic regression of heaviness of menstrual flow and menstrual cycle regularity on \( \log_{10} 2,3,7,8 \)-tetrachlorodibenzo-p-dioxin level, overall and by menarcheal status at the time of the ICMESA chemical explosion, Seveso Women’s Health Study, Italy, 1996–1998

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total no.</th>
<th>Heaviness of flow</th>
<th>Regularity of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adjusted* odds ratio† 95% confidence interval</td>
<td>Adjusted* odds ratio† 95% confidence interval</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All women</td>
<td>Premenarcheal (n = 134)</td>
</tr>
<tr>
<td>Heaviness of flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanty</td>
<td>30</td>
<td>0.84  0.44, 1.61</td>
<td>0.33  0.10, 1.06</td>
</tr>
<tr>
<td>Moderate or heavy</td>
<td>271</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>277</td>
<td>0.46  0.23, 0.95</td>
<td>0.50  0.18, 1.38</td>
</tr>
</tbody>
</table>

* Adjusted for age at interview and age at menarche.
† Change in odds with a 10-fold increase in serum 2,3,7,8-tetrachlorodibenzo-p-dioxin level (e.g., from 10 ppt to 100 ppt).
‡ Difference in the relation of the menstrual cycle characteristic to 2,3,7,8-tetrachlorodibenzo-p-dioxin level between premenarcheal women and postmenarcheal women.

p for interaction‡ = 0.03
p for interaction‡ = 0.81
tetrachlorodibenzo-\(p\)-dioxin measurements at the US Centers for Disease Control and Prevention (Atlanta, Georgia).

**REFERENCES**


