Maternal Serum Dioxin Levels and Birth Outcomes in Women of Seveso, Italy

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2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (TCDD), a ubiquitous environmental contaminant, is associated with increased fetal loss and reduced birth weight in animal studies. In 1976, an explosion at a trichlorophenol plant near Seveso, Italy, resulted in the highest TCDD exposure known in human residential populations. In 1996, we initiated the Seveso Women's Health Study, a retrospective cohort study of women who resided in the most contaminated areas, zones A and B. We examined the relation of pregnancy outcome in 510 women (888 total pregnancies) to maternal TCDD levels measured in serum collected shortly after the explosion. Ninety-seven pregnancies (10.9%) ended as spontaneous abortions (SABs). There was no association of $\log_{10}$ TCDD with SAB [adjusted odds ratio (OR) = 0.8; 95% confidence interval (CI), 0.6–1.2], with birth weight (adjusted beta = –4 g; 95% CI, –68 to 60), or with births that were small for gestational age (SGA) (adjusted OR = 1.2; 95% CI, 0.8–1.8). However, associations with birth weight (adjusted beta = –92 g; 95% CI, –204 to 19) and with SGA (adjusted OR = 1.4; 95% CI, 0.6–2.9) were stronger for pregnancies within the first 8 years after exposure. TCDD was associated with a 1.0–1.3 day nonsignificant adjusted decrease in gestational age and a 20–50% nonsignificant increase in the odds of preterm delivery. It remains possible that the effects of TCDD on birth outcomes are yet to be observed, because the most heavily exposed women in Seveso were the youngest and the least likely to have yet had a pregnancy. *Key words:* birth weight, dioxin, environmental exposures, epidemiology, small for gestational age, spontaneous abortion. *Environ Health Perspect* 111:947–953 (2003). doi:10.1289/ehp.6080 available via *http://dx.doi.org/* [Online 13 February 2003]

The compound 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD, or dioxin)—the most toxic halogenated aromatic hydrocarbon (IARC 1997)—is a ubiquitous contaminant of various industrial and combustion processes. It is highly lipophilic, is extremely stable in the environment, and thus bioaccumulates in the food chain (Birnbaum 1994). In humans, it has a half-life of 7–9 years (Pirkle et al. 1989). TCDD has been classified as a known human carcinogen (IARC 1997) and has been shown to disrupt multiple endocrine pathways (Birnbaum 1994, 1995; IARC 1997). Although health concerns of TCDD exposure initially centered on cancer, there has been growing concern about the potential developmental consequences to populations exposed to TCDD and related chemicals.

Maternal exposure to dioxin has been associated with increases in fetal loss and reduction in birth weight in experimental studies in rodents and monkeys (Allen et al. 1979; Bjerke et al. 1994; Courtney 1976; McNulty 1984; Murray et al. 1979; Nau et al. 1986; Umbreit et al. 1987). Frank structural malformations usually are not observed in animal studies, although TCDD has been associated with a developmental syndrome involving hydronephrosis, cleft palate, and fetal thymic atrophy in mice (Birnbaum and Tuomisto 2000). These developmental effects are noted at doses below those that cause overt maternal toxicity.

Case studies and anecdotal reports suggest that maternal dioxin exposure may also affect human development. For example, fetal growth retardation has been described in case reports of offspring of mothers who consumed rice oil contaminated with polychlorinated biphenyls (PCBs), dioxins, and dioxin-like furans during pregnancy in the Yusho (Yamashita and Hayashi 1985) and Yu-cheng (Guo et al. 1995) accidents (Kunita et al. 1984; Rogan 1982; Schantz 1996; Schecter et al. 1996b). Similarly, anecdotal reports of Vietnamese women residing in areas sprayed with Agent Orange, which was contaminated with dioxin, suggest an increase in spontaneous abortion (SAB), stillbirths, neural tube defects, facial clefts, and hydatidiform (molar) pregnancies. These findings have not been confirmed in well-controlled studies with exposure measurements (Constable and Hatch 1985; Le and Johansson 2001).

There are few epidemiologic studies of the association between maternal exposure to TCDD and related compounds (e.g., other dioxins, furans, and dioxin-like PCBs) and pregnancy outcomes in humans. Women in the United States who lived near a horse arena that was sprayed with dioxin-contaminated oil did not have higher rates of fetal or infant mortality, intrauterine growth retardation or lowered birth weight, or birth defects compared with unexposed women; however, exposure classification was based on soil levels around their homes (Stockbauer et al. 1988). In Chapayevsk, Russia, a town contaminated with dioxin from a chemical plant, rates of SAB, congenital defects, and preterm delivery, but not low birth weight, were higher compared with surrounding areas (Revich et al. 2001). In contrast, in Sweden, east coast fishermen's wives, who are known to consume more fish contaminated with dioxins and furans, had a higher frequency of infants with low birth weight than did west coast fishermen's wives, who consumed less contaminated fish (Rylander et al. 1995, 1996, 2000; Svensson et al. 1991).

A few studies have used biologic measures of dioxin exposure, but only in populations with low background exposures. In a Dutch population-based study of 38 infants, dioxins and furans were measured in maternal breast milk and summarized as dioxin toxic equivalents (TEQ). Birth weight and gestational age did not differ between those with breast milk TEQ ≤ 28 ppt (lipid adjusted; median = 18 ppt) and those with > 28 ppt (lipid adjusted; median = 37 ppt) (Pluim et al. 1996). A larger Dutch study (Patandin et al. 1998) reported that birth weight was negatively correlated with cord plasma PCB levels. Analysis of maternal breast milk revealed that a large proportion of total TEQ derived from dioxins, but no separate correlation between dioxins and birth weight was provided (Patandin et al. 1998). In Finland, birth weight of 166 newborns was

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negatively associated with maternal breast milk TEQ (range, 10.8–40 ppt, lipid adjusted) (Vartiainen et al. 1998).

In 1976, an explosion at a trichlorophenol plant near Seveso, Italy, resulted in the highest TCDD exposure known in human residential populations (Mocarelli et al. 1988; Needham et al. 1991). To date, few studies of the reproductive health effects of TCDD exposure in Seveso have been conducted. Until early 1978, rates of SAB were significantly elevated in the exposed zones (zones A, B, and R) relative to the control zone, zone non-ABR (Fara and Del Corno 1985). Between 1977 and 1983, no increased risk for birth defects was observed in births in any of the exposed zones, but the number of births was small, with low power to detect an effect (Mastroiacovo et al. 1988). These studies did not include any biologic measures of TCDD exposure.

Twenty years after the explosion, we initiated the Seveso Women's Health Study (SWHS), a historical cohort study, to determine whether there was an association between TCDD exposure and reproductive health. Serum samples collected soon after the explosion rendered it possible to quantify individual TCDD exposure (Mocarelli et al. 1990). Because the half-life of TCDD in humans is very long (Pirkle et al. 1989), a preconception exposure of a woman could result in *in utero* exposure to her fetus conceived many years later. We examined the relationship of maternal serum TCDD levels and risk of adverse pregnancy outcome in postexplosion pregnancies, including SAB, congenital anomalies and disorders, lowered birth weight, and shortened gestational age.

Materials and Methods

Study population. The explosion at the ICMESA chemical factory near Seveso, Italy, located approximately 25 km north of Milan, occurred on 10 July 1976. Up to 30 kg of TCDD was deposited over an 18 km^2 area (Di Domenico et al. 1980). The area was subsequently divided into exposure zones based on surface soil TCDD measurements. Zone A was the most heavily contaminated area, and zone B was the next heaviest contaminated area (Mocarelli et al. 1992). As part of a health assessment, blood samples were collected for clinical chemistry tests from residents soon after the explosion; the remaining portion of the serum was stored for future studies (Mocarelli et al. 1988).

The follow-up study of the women was conducted in 1996–1998, approximately 20 years after the explosion. Women eligible for the SWHS were 40 years old or younger in 1976, had adequate stored sera collected between 1976 and 1980, and had resided in zone A or B at the time of the explosion. A total of 1,271 women met these criteria. Seventeen women could not be contacted, and 33 had died or were too ill to participate. Of the 1,221 women contacted, 981 (80%) agreed to participate. Informed consent was obtained from all women before participation. Of the 981 women in SWHS, 745 reported having been pregnant, with a total of 1,822 pregnancies. Of these, 888 pregnancies in 510 women were completed after the explosion, including 33 pregnancies conceived before the explosion. At follow-up, the 510 women who had a postexplosion pregnancy were younger at interview (mean ± SD = 39.7 ± 8.0 years) than the entire cohort (mean ± SD = 40.8 ± 11.7 years) but older than the 236 women who had not yet had a pregnancy (mean ± SD = 29.2 ± 8.1 years). The women who had a postexplosion pregnancy at the time of follow-up had lower serum TCDD levels [median = 46.2 ppt; interquartile range (IQR) = 24.3–104.0] than those women who had not been pregnant (median = 142.5 ppt; IQR = 52.3–304.5).

The analysis of SAB is restricted to the 769 pregnancies (476 women), including 13 multiple births, that did not end in voluntary abortion (n = 108), ectopic pregnancy (n = 10), or molar pregnancy (n = 1). We describe congenital anomalies/disorders among those pregnancies that did not end in SAB (n = 672 pregnancies, 443 women). The analysis of fetal growth and gestational age is limited to the 608 singleton postexplosion live births (414 women). For the fetal growth and gestational age analyses, we excluded women with hypertensive disorders of pregnancy (n = 42) or diabetes (n = 5).

Procedure. Details of the study procedure are presented elsewhere (Eskenazi et al. 2001). 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. We derived the information used for analyses from the woman's report of her pregnancy history. For each pregnancy, the woman was asked to indicate the outcome of the pregnancy (singleton live birth, multiple birth, SAB, ectopic pregnancy, molar pregnancy, voluntary abortion, stillbirth, current pregnancy); the date the pregnancy ended; and the length of pregnancy (in weeks or months or if unknown, whether it was full term). For all live births, the woman was asked the birth weight in grams, the child's sex, and whether or not the child had any birth defects or other congenital or developmental diseases. Medical records were requested for all spontaneous and voluntary abortions, although not all medical records were located and not all outcomes were confirmed. We did not request birth certificates to confirm birth weight or gestational age, because in Italy the certificates do not contain this information. Studies have suggested that maternal report of birth weight is accurate when compared with medical records and birth certificates (Selevan 1980); nevertheless, to validate a portion of reports, we requested all medical records for cesarean section deliveries and for deliveries occurring at the central hospital (Hospital of Desio) (n = 139/601 live births = 23%).

Laboratory analyses. For each participant, we selected the first serum sample collected between 1976 and 1981 and of adequate volume (> 0.5 mL) for analysis. Sodium concentration was measured to check for desiccation before determining individual lipid levels (total cholesterol and triglycerides). The TCDD concentration in these samples, which had been stored at –20°C at Desio Hospital, Italy, was measured by high-resolution mass spectrometry methods at the U.S. Centers for Disease Control and Prevention (Patterson et al. 1987). Values were reported on a lipid-weight basis in parts per trillion (Akins et al. 1989).

TCDD was measured in sera collected in 1976 or 1977 for 413 women (81%), from 1978 through 1981 for 12 women (2%), and in 1996 for 19 women (4%) whose earlier samples had become concentrated by desiccation. For women with post-1977 TCDD values that were detectable but ≤ 10 ppt (n = 6), the measured value was used. For women with post-1977 TCDD levels > 10 ppt, the TCDD exposure level was back-extrapolated to 1976 using the Filser model, a physiologically based toxicokinetic model (Kreuzer et al. 1997), for women 16 years old or younger in 1976 (n = 10), and the first-order kinetic model for older women (n = 15) (Pirkle et al. 1989). For nondetectable values (n = 66, 13%), a serum TCDD level equal to one-half the detection limit was assigned (median detection limit = 18.8 ppt) (Hornung and Reed 1990).

Statistical analyses. Statistical analyses were conducted using STATA 7.0 (Stata 2001). We analyzed the data to examine all pregnancies that ended between the explosion in 1976 and time of interview (1996–1998) and all pregnancies occurring within the first 8 years (1976–1984) or approximately the first half-life after the explosion (Pirkle et al. 1989), when exposure body burden would be greatest. Because pregnancy and/or lactation may affect the TCDD levels in later pregnancies (Abraham et al. 1996, 1998; Schecter 1998; Schecter et al. 1996a), we also analyzed the subset of data including only the first postexplosion pregnancies occurring within the two time frames (1976–1998 and 1976–1984).

The log (base 10) of serum TCDD was entered as a continuous variable into the regression equations. A one-unit change in $\log_{10}$ TCDD corresponds to a 10-fold increase in TCDD itself; therefore, reported regression

effects (coefficients, odds ratios) are those associated with a 10-fold increase in TCDD. We used logistic regression to examine the relation of TCDD to SAB, preterm delivery (< 37 weeks gestation), and small for gestational age [SGA; defined as less than the 10th percentile of weight by sex at a given gestational age based on population statistics for northeast Italy (Parazzini et al. 1995)]. For these outcomes, we report the odds ratio (OR) and 95% confidence intervals (CIs). We examined the relation of serum TCDD levels to birth weight in grams and to gestational age in days as continuous end points using least-squares linear regression models; we report the coefficient (beta) and 95% confidence intervals (95% CIs). Because of strong digit preference—namely, reports of birth weight as multiples of 100 g and of gestational age in whole weeks—we used options in STATA to yield robust standard errors. For analyses with multiple pregnancies per woman, we used "cluster" options in STATA to account for nonindependence.

We selected potential confounders and effect modifiers *a priori* from the literature. These covariates included maternal age at pregnancy, education at interview, maternal smoking, maternal alcohol use, previous parity, history of low birth weight, history of SAB, body mass index (BMI; in kilograms per square meter), height, maternal weight gain, gestational age, infant's sex, and years from pregnancy to interview. BMI was based on the height and weight measured at interview. The values of other covariates at the time of pregnancy were determined from the interview histories. Covariates that confounded the relationship of TCDD levels and the outcome in bivariate analyses (defined as a > 10% change in the coefficient for TCDD) were entered into a multivariate model and tested by likelihood ratio. For continuous outcomes, we also included an interaction term for TCDD and sex.

If TCDD affected the pregnancy outcome, the relevant body burden might be either the initial dose (if this produced permanent perturbations of the reproductive system) or the body burden at the time of the pregnancy. Hence, we repeated all final models with body burden of TCDD estimated at the time of pregnancy. We estimated TCDD at the time of pregnancy by extrapolating from the TCDD level measured in serum near the explosion to the date of the pregnancy using the same models described above (Kreuzer et al. 1997; Pirkle et al. 1989). Specifically, for women (n = 73) with pregnancies that occurred before 31 December 1977, we used the mother's serum TCDD value at the time of the explosion. For women (n = 189) who were 16 years old or younger in 1976, we used the Filser model to extrapolate from serum TCDD level near the time of the explosion to the time of her first postexplosion pregnancy and then used the first-order kinetic model for later pregnancies. For women (n = 248) who were older than 16 years at the time of explosion, we used exclusively the first-order kinetic model to estimate serum TCDD levels at all postexplosion pregnancies.

Results

Table 1 presents sociodemographic characteristics, pregnancy-related factors, and TCDD levels for the 510 women and their 888 postexplosion pregnancies. The average age for the women at the time of explosion was 19.1 years old (SD = 7.9) and at interview was 39.7 years old (SD = 8.0). Most women (71%) were nulliparous at the time of explosion. The mean age at their postexplosion pregnancy was 28.6 years (SD = 5.4). The average length of recall between interview and pregnancy was 10.7 years (SD = 6.2). At interview, 23% of women were overweight and 66.5% had completed only high school. For the time during the pregnancy, 12% reported smoking and most reported gaining 10–15 kg.

The median maternal serum TCDD level at the time of the explosion was 46.6 ppt, with an interquartile range of 24.3–104.0 ppt (range, 2.5–9,140 ppt). TCDD levels were highest for the youngest group of women, women who were nulliparous at the time of the explosion, and for women with the shortest time of recall to their pregnancy.

Table 1. Selected sociodemographic and pregnancy characteristics by maternal serum TCDD for 510 women representing 888 pregnancies.

Characteristics	No. (%)	Serum TCDD [ppt; median (IQR)]
Total women	510 (100)	46.6 (24.3–104.0)
Age at explosion (years)		
0–10	62 (12.2)	70.4 (33.3–196.0)
11–20	211 (41.4)	49.9 (23.5–102.0)
21–30	180 (35.3)	38.8 (20.9–79.0)
31–40	57 (11.2)	39.9 (28.5–92.7)
Age at interview (years)		
20–29	37 (7.3)	75.2 (43.7–214.0)
30–39	228 (44.7)	51.3 (24.4–111.5)
≥ 40	245 (48.0)	40.6 (22.9–81.7)
Preexplosion parity*		
0	362 (71.0)	52.6 (25.5–120.0)
1	78 (15.3)	37.6 (21.1–72.1)
≥ 2	70 (13.7)	36.5 (27.3–67.9)
Maternal education at interview		
< Required	155 (30.4)	40.4 (23.3–86.2)
Required/high school	339 (66.5)	49.9 (25.4–115.0)
University	16 (3.1)	39.1 (13.4–181.3)
Maternal BMI (kg/m^2) at interview		
Underweight (< 19.8)	66 (12.9)	61.2 (33.0–129.0)
Normal (19.8–26.0)	329 (64.5)	45.8 (22.1–74.7)
Overweight (26.1–29.0)	65 (12.8)	42.1 (22.1–74.7)
Obese (> 29.0)	50 (9.8)	37.4 (14.0–112.0)
Total pregnancies	888 (100)	46.6 (24.3–104.0)
Age at pregnancy (years)*		
< 20	33 (3.7)	43.1 (13.2–84.8)
20–24	163 (18.4)	45.6 (21.6–105.0)
25–29	343 (38.6)	53.7 (26.6–104.0)
> 29	349 (39.3)	41.9 (23.2–88.5)
Year of pregnancy*		
1976–1984	402 (45.3)	38.4 (21.1–80.9)
1985–1993	360 (40.5)	52.6 (26.2–100.0)
1994–1997	126 (14.2)	53.2 (27.0–135.0)
Years recalled to pregnancy*		
< 5	188 (21.2)	53.5 (27.3–119.0)
5–10	235 (26.5)	52.5 (27.6–119.0)
11–15	218 (24.6)	44.8 (22.5–98.3)
> 15	247 (27.8)	37.2 (20.6–72.1)
Infant sex[a]		
Male	317 (52.9)	44.9 (22.5–88.5)
Female	282 (47.1)	59.7 (25.1–122.0)
Smoking status at pregnancy*[a]		
Nonsmoker	530 (88.2)	52.6 (25.1–110.5)
Smoker	71 (11.8)	38.0 (16.3–76.2)
Maternal weight gain (kg)*[a]		
< 10	121 (20.2)	66.2 (35.6–119.0)
10–15	333 (55.6)	45.8 (22.0–92.4)
16–20	96 (16.0)	49.6 (19.5–122.0)
> 20	25 (4.2)	47.1 (23.5–92.9)

[a]Live births only. *ANOVA for $\log_{10}$ TCDD, $p < 0.05$.

Among the 888 postexplosion pregnancies were 655 (73.8%) singleton live births, 13 (1.5%) multiple births, 97 (10.9%) SABs, 4 (0.4%) stillbirths, 10 (1.1%) ectopic pregnancies, 1 (0.1%) molar pregnancy, and 108 (12.2%) voluntary abortions.

SAB. The 97 SABs terminated, on average, at 9.6 weeks gestation (SD = 4.0 weeks). Women who were older at the time of pregnancy, were nulliparous, or had less than the required schooling were more likely to have had an SAB (data not shown).

Figure 1A presents the cumulative distribution of TCDD levels for mothers of SAB cases and for live births. The distribution of the SAB cases is slightly shifted to the left, implying slightly lower levels of TCDD in mothers of SAB cases than in mothers of live births (median TCDD = 37.5 ppt and 48.6 ppt, respectively). Table 2 presents the ORs for SAB and a 10-fold increase in TCDD, both unadjusted and adjusted for maternal age, education, and history of SAB before the explosion.

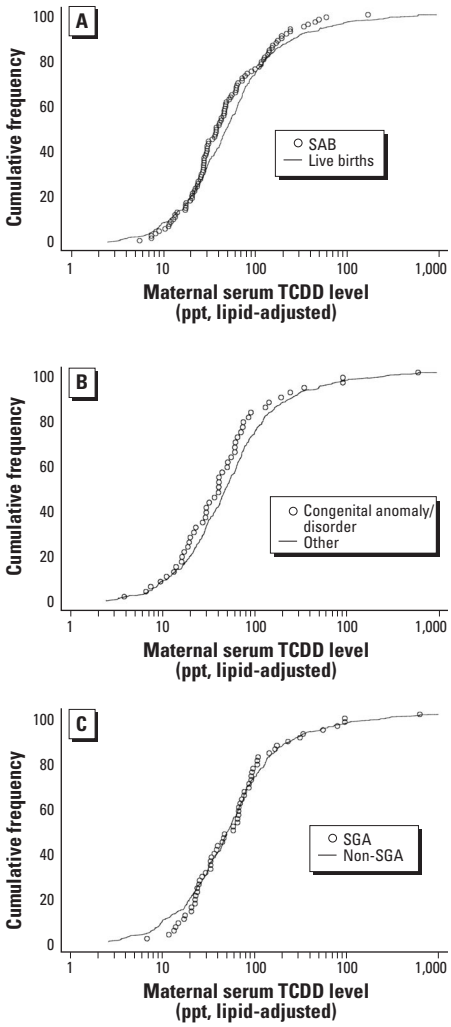

Figure 1. Cumulative distributions of maternal serum TCDD levels by (*A*) SAB, (*B*) congenital anomalies/disorders, and (*C*) SGA status.

There was no increase in the OR for SAB for the 769 pregnancies that occurred between 1976 and 1998 (adjusted OR = 0.8; 95% CI, 0.6–1.2) or among the 343 pregnancies that occurred within the first half-life of TCDD (1976–1984) (adjusted OR = 1.0; 95% CI, 0.6–1.6). There also was no association between TCDD and SAB when the analysis was limited to the 467 first postexplosion pregnancies occurring from 1976 to 1998 (adjusted OR = 0.8; 95% CI, 0.5–1.3) or to the 251 first postexplosion pregnancies occurring from 1976 to 1984 (adjusted OR = 0.9; 95% CI, 0.4–1.9). When we restricted these analyses to the 70 SAB cases that were confirmed by medical records, the results were similar (data not shown). Substitution of TCDD level extrapolated to the time of the conception did not appreciably alter any of these findings.

In addition to SAB, there were other adverse pregnancy outcomes reported by mothers. There were 10 ectopic pregnancies (median TCDD = 51.6 ppt; range, 11.5–1,420.0), four stillbirths (median TCDD = 34.8 ppt; range, 23.3–3,690.0), one molar pregnancy (median TCDD = 61.1 ppt), and one neonatal death due to asphyxia (maternal serum TCDD = 29.9 ppt).

Congenital anomalies or disorders. The number (n = 46) of any one specific congenital anomaly or disorder reported by the mothers is small and was not analyzed statistically. Figure 1B indicates that the maternal serum TCDD levels for births with congenital anomalies/disorders were slightly lower than those for births without (median TCDD = 40.6 ppt and 49.5 ppt, respectively). There was one case of anencephaly and one case of cleft lip in a child who had toxoplasmosis; both mothers had serum TCDD levels below the median for the population, 19.5 and 29.9 ppt, respectively. There were also two cases of hypospadias or epispadias; both mothers had serum TCDD levels slightly above the median for the population, 61.2 and 74.7 ppt, respectively. On the basis of congenital anomaly registry data from a nearby area of Italy (ICBDMS 1999), we expected 0.02 cases of anencephaly (observed = 1), 0.33 cases of cleft lip (observed = 1), and 0.26 cases of hypospadias (observed = 2). A few cases of congenital/developmental anomalies or disorders were reported by mothers with TCDD levels > 100 ppt, including sleep apnea (n = 1), pyloric stenosis (n = 1), von Recklinhausen's disease (n = 1), syndactaly (n = 1), histiocytosis (n = 1), ventricular tachycardia (n = 1), hypoplasia of the cord (n = 1), and thallassemia minor (n = 2).

Birth weight, gestational age, and SGA. Birth weight and gestational age results are presented for the 608 singleton births (414 women). Mean birth weight was 3,281 g (SD = 480 g), with a low-birth-weight rate (< 2,500 g) of 5.1%. The mean gestational age was 39.4 weeks (SD = 1.7 weeks), with a preterm delivery rate of 4.9%. There were 59 (9.7%) SGA infants. Average birth weights tended to be lower among infants of women who were underweight, were nulliparous, or reported less weight gain during pregnancy (data not shown). Shortened gestational age was associated with a woman's report of less weight gain during pregnancy.

Table 3 summarizes the crude and adjusted linear regression results for birth weight and TCDD. Unadjusted and adjusted results were similar. A 10-fold increase in the maternal serum TCDD levels was not associated with a change in birth weight among all postexplosion pregnancies (1976–1998) (adjusted beta = –4 g; 95% CI, –68 to 60) or among the first postexplosion pregnancy (adjusted beta = –34 g; 95% CI, –99 to 31) after controlling for maternal age, education, smoking during pregnancy, gestational age, sex of infant, parity, BMI at interview, and history of a low-birth-weight infant before the explosion. However, there were stronger, albeit not significant, associations of lowered birth weight with TCDD among pregnancies occurring in the first half-life of TCDD (1976–1984; all pregnancies: adjusted beta = –92 g; 95% CI, –204 to 19; first postexplosion pregnancy: adjusted beta = –89 g; 95% CI, –203 to 25).

Figure 1C shows that the cumulative distributions of maternal serum TCDD were similar for SGA and non-SGA infants (median TCDD = 55.0 ppt and 50.2 ppt, respectively). After adjusting for parity, history of low birth weight, maternal height and BMI, age, education, and smoking status, a 10-fold increase in TCDD was associated with a nonsignificant increase in odds of an SGA infant among all postexplosion pregnancies (adjusted OR = 1.2; 95% CI, 0.8–1.8) and among the first postexplosion pregnancy (adjusted OR = 1.5; 95% CI, 0.9–2.6; Table 3). These associations, like those for birth weight, were stronger but still statistically nonsignificant when we limited the

Table 2. Crude and adjusted ORs and 95% CIs for SAB per 10-fold increase in maternal serum TCDD level.

			Log_{10} TCDD [n (%)]		
Pregnancies	Years	No.	SAB	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
All eligible	1976–1998	769	97 (12.6)	0.8 (0.6–1.1)	0.8 (0.6–1.2)
	1976–1984	343	44 (12.8)	1.0 (0.6–1.6)	1.0 (0.6–1.6)
First eligible	1976–1998	467	55 (11.8)	0.8 (0.5–1.3)	0.8 (0.5–1.3)
	1976–1984	251	31 (12.4)	0.9 (0.4–1.9)	0.9 (0.4–1.9)

[a]Adjusted for maternal age, maternal education, and history of SAB.

analysis to pregnancies within the first half-life of TCDD (1976–1984; all pregnancies: adjusted OR = 1.4; 95% CI, 0.6–2.9; first postexplosion pregnancies: OR = 1.8; 95% CI, 0.7–4.3). The results were similar when estimated TCDD level at the time of the pregnancy was substituted for the measured value near the time of the explosion.

Table 4 presents the relationship of serum TCDD levels and gestational age and preterm delivery. Results for crude and adjusted analysis are similar. There is about a 1.0–1.3-day nonsignificant decrease in gestational age per 10-fold increase in maternal serum TCDD and a 20–50% nonsignificant increase in odds of preterm delivery after controlling for potential confounders, regardless of the subsample examined. When we substituted estimated TCDD at the time of the pregnancy for measured TCDD, the results were unchanged.

Discussion

The SWHS of female residents living near Seveso in 1976 at the time of the explosion did not find profound effects of TCDD on pregnancy outcomes, including SAB, congenital anomalies/disorders, and measures of birth weight and gestational duration, when TCDD exposure was determined before conception from blood collected from mothers soon after the explosion or extrapolated to the time of the conception. However, the associations with serum TCDD levels were somewhat stronger, albeit nonsignificant, for lowered birth weight and the occurrence of SGA infants in pregnancies that ended within the first half-life of TCDD after the explosion.

Our results differ from those in the literature that have reported higher rates of SAB and fetal growth retardation in monkeys and rodents (Allen et al. 1979; Bjerke et al. 1994; Courtney 1976; McNulty 1984; Murray et al. 1979; Nau et al. 1986; Umbreit et al. 1987). Our results also differ from case reports in the Yusho (Yamashita and Hayashi 1985) and Yu-cheng populations (Guo et al. 1995), in Vietnamese residents (Constable and Hatch 1985; Le and Johansson 2001), and in epidemiologic studies of populations with background exposure to dioxin-like chemicals (Patandin et al. 1998; Rylander et al. 1995; 2000; Svensson et al. 1991; Vartiainen et al. 1998). Only a few studies have documented the levels and the exact nature of exposure of the persons studied (Patandin et al. 1998; Pluim et al. 1996; Vartiainen et al. 1998). Thus, many of these populations may have differed in the mixture of dioxins, furans, and PCBs to which they were exposed, which could explain differences in study results. In the Seveso study, we did not measure levels of other dioxin-like compounds [polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDFs), PCBs], because the small volumes of the archived serum (averaging 0.6 mL) did not permit the additional analyses. We presumed that exposure was predominantly to TCDD, the specific dioxin-like toxicant released in the explosion, and that other compounds were at background levels.

Our study has some important limitations. We were not able to confirm all the pregnancy outcomes with medical records, but instead based our results on maternal report. Rasmussen et al. (1990) found that women tended to underreport the presence of birth defects in their children, but few reported a defect when none was noted on the medical records. However, these authors found no false reports for neural tube defects, hypospadias, and cleft palate, the defects of greatest concern based on case reports of TCDD-exposed women (Constable and Hatch 1985). Other studies have suggested that women are accurate reporters of their children's birth weight (Axelsson and Rylander 1984; Burns et al. 1987). In birth weights confirmed by medical records for the subset of SWHS infants (n = 139) delivered by cesarean section or at the Hospital of Desio, we found that women overestimated their infant's birth weight by 22 g, although there was no difference in the median birth weights obtained by report and by records (range = –510 to 700 g).

We were not able to ascertain the outcomes of all possible pregnancies. Many women in Seveso may have chosen to have a voluntary abortion after the explosion because of concerns about the risk to their fetus. In fact, almost one-third of pregnancies ended in voluntary abortion within the first year after the explosion, a rate that fell to an average of 11% thereafter. A proportion of these pregnancies could have resulted in an adverse outcome (Susser 1983). However, the voluntary abortion rate did not vary by TCDD exposure (data not shown). Therefore, the high initial rate of voluntary abortions probably did not bias the study findings. Also, we ascertained only clinically recognized SABs and not those losses that occurred before a woman was aware that she was pregnant. If TCDD exposure acted to increase preclinical loss, we would have missed this effect entirely (Eskenazi et al. 1995; Wilcox et al. 1988), and thus we would have underestimated the impact of TCDD exposure.

Although we did not observe an association between TCDD and adverse pregnancy outcome, it is biologically plausible. Dioxins are known to cross the placenta and have been measured in follicular fluid in humans (Tsutsumi et al. 1998). TCDD accelerates blastocyst formation and cell number in mouse, which may be related to the cellular proliferation associated with cleft palate. TCDD may exert some of its developmental effects by modulating the stimulatory effect of

Table 3. Crude and adjusted β and 95% CIs for birth weight and OR for SGA infants per 10-fold increase in maternal serum TCDD levels (log_{10} TCDD).

		Birth weight (g)			SGA		
Pregnancies	Years	No.	Crude β (g) (95% CI)	Adjusted β (g) (95% CI)[a]	No. (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
All eligible	1976–1998	608	–33 (–108 to 43)	–4 (–68 to 60)	59 (9.7)	1.1 (0.7–1.7)	1.2 (0.8–1.8)
	1976–1984	275	–96 (–226 to 35)	–92 (–204 to 19)	28 (10.2)	1.2 (0.6–2.5)	1.4 (0.6–2.9)
First eligible	1976–1998	414	–58 (–136 to 20)	–34 (–99 to 31)	43 (10.4)	1.4 (0.8–2.2)	1.5 (0.9–2.6)
	1976–1984	221	–91 (–222 to 40)	–89 (–203 to 25)	24 (10.9)	1.5 (0.6–3.3)	1.8 (0.7–4.3)

[a]Adjusted for gestational age, gestational age squared, sex of infant, parity, history of low-birth-weight infant, maternal height, maternal BMI, maternal age, maternal education, maternal smoking status at pregnancy. [b]Adjusted for parity, history of low-birth-weight infant, maternal height, maternal BMI, maternal age, maternal education, maternal smoking status at pregnancy.

Table 4. Crude and adjusted β and 95% CIs for gestational age and ORs for preterm delivery per 10-fold increase in maternal serum TCDD levels (log_{10} TCDD).

		Gestational age (days)			Preterm delivery (< 37 completed weeks)		
Pregnancies	Years	No.	Crude β (days) (95% CI)	Adjusted β (days) (95% CI)[a]	No. (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
All eligible	1976–1998	608	–1.1 (–2.9 to 0.7)	–1.2 (–2.9 to 0.5)	30 (4.9)	1.2 (0.7–2.4)	1.3 (0.7–2.3)
	1976–1984	275	–0.7 (–3.0 to 1.7)	–1.0 (–3.1 to 1.2)	15 (5.5)	1.1 (0.4–2.8)	1.5 (0.7–3.2)
First eligible	1976–1998	414	–0.9 (–2.7 to 1.0)	–1.0 (–2.7 to 0.8)	21 (5.1)	1.2 (0.6–2.4)	1.2 (0.6–2.5)
	1976–1984	221	–1.0 (–3.9 to 1.8)	–1.3 (–4.0 to 1.3)	13 (5.9)	1.4 (0.5–4.0)	1.5 (0.5–4.8)

[a]Adjusted for sex of infant, parity, history of low-birth-weight infant, maternal height, maternal BMI, maternal age, maternal education, maternal smoking status at pregnancy. [b]Adjusted for parity, history of low-birth-weight infant, maternal height, maternal BMI, maternal age, maternal education, maternal smoking status at pregnancy.

epidermal growth factor (EGF). In placentas of eight Yu-cheng women exposed to PCBs and PCDFs, EGF-stimulated receptor autophosphorylation was significantly decreased, compared with placentas of unexposed women, and this decrease in turn was correlated with lower birth weight (Lucier et al. 1990; Sunahara et al. 1987). The fetal growth retardation effects of TCDD in experimental animals (mice) have been hypothesized to be caused by TCDD-induced production of reactive oxygen species and DNA damage in fetal and placental tissues (Hassoun et al. 1997). Most studies are consistent with the hypothesis that the effects of TCDD are mediated via binding to the aryl hydrocarbon (Ah) receptor. The Ah receptor mechanism may be involved in antiestrogenic effects that may play a role in the developmental and reproductive effects of TCDD observed in animals (Peterson et al. 1993). However, differences in susceptibility across experimental animal strains and species make it difficult to extrapolate to human pregnancy.

Although it is biologically plausible that maternal exposure to TCDD could have an impact on the developing fetus, there is some evidence that the most profound effects on the fetus may be paternally rather than maternally (as presented here) mediated. For example, Mocarelli et al. (1996, 2000) reported in the Seveso cohort an increased probability of female births associated with paternal exposure but not with maternal exposure. In the subset of the Seveso cohort who participated in SWHS, we also did not observe a shift in sex ratio associated with maternal exposure (Table 1). Only a small proportion of the fathers of the SWHS pregnancies were likely to have substantial exposure to TCDD. Mothers reported that at the time of explosion fewer than 10% of the fathers were residents of zone A, about a third were from zone B, and more than half were from nonexposed areas.

In conclusion, we report the lack of an association between maternal serum levels of TCDD and adverse birth outcomes in the cohort of women exposed to dioxin in Seveso, Italy. However, associations of TCDD and lowered birth weight and SGA are somewhat stronger, albeit nonsignificant, for those pregnancies occurring in the first TCDD half-life (within 8 years) after the explosion. It remains possible that the effects of TCDD on birth outcomes are yet to be observed, because the most heavily exposed women were the youngest at follow-up and therefore less likely to have yet had a postexplosion pregnancy (Eskenazi et al. 2001).

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