Spontaneous Abortion, Sex Ratio, and Paternal Occupational Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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There is conflicting research regarding an association between fetal death and paternal exposure to Agent Orange, a phenoxy herbicide widely used in Vietnam that was contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Men who worked in the U.S. factories that produced Agent Orange were exposed to TCDD at levels hundreds of times higher than TCDD levels in the general population. Wives of TCDD-exposed chemical workers and wives of nonexposed neighborhood referents were interviewed to determine reproductive history. Paternal serum TCDD level at time of conception was estimated for each pregnancy using serum samples taken in 1987. Estimated TCDD levels of workers during or after exposure were high (median, 254 ppt; range, 3–16,340 ppt) compared to referent levels (median, 6 ppt; range, 2–19 ppt). No association between paternal TCDD level at the time of conception and spontaneous abortion was observed among pregnancies fathered by workers with TCDD levels of < 20 ppt [odds ratio (OR) = 0.77; 95% confidence interval (CI), 0.48–1.22], 20 to < 255 ppt (OR = 0.81; 95% CI, 0.40–1.63), 255 to < 1,120, (OR = 0.69; 95% CI, 0.30–1.58), and ≥ 1,120 ppt (OR = 0.95; 95% CI, 0.42–2.17) compared to pregnancies fathered by referents. The sex ratio [males/(males + females)] of offspring also did not differ by TCDD exposure (0.53 and 0.54 among workers and referents, respectively). We did not find an association between paternal serum TCDD level and spontaneous abortion or sex ratio of offspring in this population. The estimated TCDD levels in this exposed worker population were much higher than in other studies, providing additional evidence that paternal TCDD exposure does not increase the risk of spontaneous abortion at levels above those observed in the general population. The study could not evaluate the effect of father's childhood or prenatal TCDD exposure on subsequent sex ratio. *Key words*: 2,3,7,8-TCDD, dioxin, occupation, paternal exposure, serum dioxin levels, sex ratio, spontaneous abortion. *Environ Health Perspect* 109:1127–1132 (2001). [Online 22 October 2001] *http://ehpnet1.niehs.nih.gov/docs/2001/109p1127-1132schnorr/abstract.html*

The National Academy of Sciences (*1*) has concluded that although there is limited suggestive evidence of an association between paternal exposure to phenoxy herbicides and spina bifida, there is inadequate or insufficient evidence to determine if an association exists between paternal exposure to the herbicides and other adverse reproductive and developmental outcomes such as fetal death. Of particular interest is the phenoxy herbicide Agent Orange, a mixture of the herbicides 2,4-D [(2,4-dichlorophenoxy)acetic acid] and 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid]. Agent Orange was widely used as a defoliant in Vietnam and was contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Recently, the U.S. Environmental Protection Agency produced a draft document assessing risks to the U.S. general population from exposure to TCDD and other dioxins in the general environment (*2*).

Most studies of reproductive effects in humans have been limited to date by small sample size and insufficient exposure assessment. Studies of fetal loss due to paternal workplace exposure to Agent Orange and other chemicals contaminated with TCDD have reported no association (*3*–*5*), and studies of Vietnam veterans thought to be exposed to Agent Orange have shown conflicting results (*6*–*9*). One large study that estimated exposure by measuring serum TCDD levels in Vietnam veterans exposed to Agent Orange found a suggestion of an increased risk of spontaneous abortion among the wives or partners of veterans exposed to low or background level and no increased risk in the highly exposed group (*8*).

A relationship between paternal TCDD level and lower sex ratio [males/(males + females)] of their offspring was reported in a population exposed to TCDD after an industrial accident in Seveso, Italy. The effect was most pronounced among men who had been exposed when they were younger than 18 years of age (*10,11*). However, a change in sex ratio was not observed among offspring of men who sprayed Agent Orange and other dioxin-contaminated herbicides in Vietnam (*12*).

We studied the pregnancy outcomes among wives of male chemical workers who were exposed to chemicals contaminated with TCDD at plants in New Jersey and Missouri and among nonexposed neighborhood referents that participated in a cross-sectional medical study. A previous analysis of the men in this study population found subtle alterations in male reproductive gonadotrophin and testosterone levels associated with TCDD serum levels (*13*). In the current study, we examined whether paternal exposure to TCDD at the time of conception was associated with spontaneous abortion or altered sex ratio in a highly exposed population for whom we had serum TCDD measurements.

Materials and Methods

The current reproductive health study was executed as part of a cross-sectional medical study conducted by the National Institute for Occupational Safety and Health (NIOSH). The details of the cross-sectional medical study design have been described previously (*14,15*). The medical study, conducted in 1987–1988, examined 281 workers from two plants. Workers were exposed to TCDD during the production of sodium trichlorophenol or one of its derivatives, such as hexachlorophene [2,2′-methylene-bis-(3,4,6-trichlorophenol)] or 2,4,5-T, which was used to formulate Agent Orange. Three hundred twenty-five referents with no self-reported occupational exposure to TCDD-contaminated substances were selected from the workers' neighborhoods at the time of the study and matched on age (± 5 years), race, and sex. Information on health status and risk factors was obtained via questionnaire and medical examination, which also included drawing blood for determination of serum TCDD. The methods of serum collection, analytical techniques, and quality control standards of the laboratory analyses have been presented elsewhere (*16*–*20*). Informed consent was obtained from all study subjects.

For the reproductive health study, trained interviewers administered a brief questionnaire on reproductive history to the male study subjects, including contact information for current and former wives/partners (hereafter referred to as "wives").

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In-depth telephone interviews with the wives of the study subjects collected detailed information on reproductive history, medical history, lifestyle factors, and occupational factors. Data for the 14 women who worked at the plants and their referents were not included in the analysis due to the small number of pregnancies.

Exposure assessment. For workers, all pregnancies conceived after the first date of the father's exposure at the plant were considered exposed. Dates of employment in dioxin-related processes defined the exposure period (*21*). Pregnancies conceived before the father's exposure at the plant were considered unexposed. For referents, all pregnancies were considered unexposed.

For workers, we used company work-history records and paternal serum TCDD levels to classify TCDD exposure level for each pregnancy. The worker's serum TCDD level at the time of each conception was estimated using a pharmacokinetic model (*22*). The TCDD hepatic elimination rate constant for humans in this model was estimated by Thomaseth and Salvan (*23*) as 0.022 $days^{-1}$. The pharmacokinetic model used to estimate serum TCDD levels at the time of conception was based on the following factors: serum TCDD level at time of examination, dates of employment in dioxin-related processes, body mass index (BMI) measured by NIOSH at the time of examination, and BMI measured by the employer during employment. BMI change over time was modeled as a continuous function of age (*23*). We used data for workers who had both BMI values to create a linear regression model of BMI change over time using age at first employment, age at examination, and BMI at examination. This model was used to derive a BMI value at time of first employment for those workers with a missing BMI value ($n = 23$).

One advantage of this modeling technique is that it allowed for changes in individual body burden over time. The model assumes a steady rate of background exposure to TCDD for workers, which was estimated using the measured serum TCDD values in the referents. We estimated individual occupational TCDD exposure rates for each exposed worker based on their final measured serum TCDD concentration and their dates of exposure in TCDD-related processes. It was also assumed that there was no occupational exposure to TCDD after termination of work; however, the background exposure to TCDD was assumed to continue throughout life. Figure 1 illustrates the estimated serum TCDD levels over time for one study subject employed at one of the study plants. The TCDD levels increase from first exposure to last exposure and then gradually decline to the level measured at the examination. The TCDD level at the times of conception for five pregnancies fathered by this worker are indicated on the graph. We did not estimate a TCDD level for pregnancies fathered by workers who had a TCDD level < 10 ppt at the time of the examination because, at these low levels, it is unclear whether TCDD serum level at examination was lower than in the past due to TCDD elimination over time or higher due to cumulative background environmental exposures ($n = 44$). Instead, we assigned the TCDD serum value measured at the time of his examination to these pregnancies. Pregnancies fathered by workers before exposure were assumed to have the same background exposure as the referents and so were assigned the median referent serum TCDD level of 6 ppt (*21,24*).

TCDD serum measurements were obtained for a random sample of 79 referents at examination. Because the referent TCDD serum level at examination was assumed to be the cumulative level from lifetime background environmental exposures, we assigned the TCDD serum value measured at the time of examination to the pregnancies fathered by these referents. For the remaining referents whose serum TCDD was not measured, the median referent value of 6 ppt was assigned (*21,24*).

Definition of a study pregnancy. Information on pregnancies and their outcomes was obtained by self-report from the wives of the study subjects. Each singleton conceptus fathered by a study subject was classified into three pregnancy outcomes of interest: spontaneous abortion, stillbirth, and live birth. A spontaneous abortion was defined as a pregnancy that was involuntarily terminated ≤ 20 weeks from the last menstrual period. A stillbirth was defined as an infant born after the 20th week of gestation showing no signs of life. All other pregnancy outcomes were excluded (ectopic/tubal pregnancies, induced abortions, pregnancies that were current at the time of the interview, and twin pregnancies). Pregnancies exposed to contraceptive methods such as birth control pills, intrauterine devices, or injections to induce a menstrual period were also excluded due to the high rate of spontaneous abortion associated with these factors. In addition, stillbirths resulting from the incompatibility of the Rh factor were excluded (Figure 2).

Statistical analysis. Univariate analyses using SAS GENMOD with repeated measures (SAS Institute, Cary, NC) were used to search for medical, exposure, and lifestyle factors that could potentially confound multivariate analyses. A variable was considered a potential confounder and retained for further modeling if it was significantly related to both TCDD level and pregnancy outcome ($p < 0.25$) or changed the odds ratio by more than 15%. Two main analyses characterized pregnancies by TCDD level at the time of conception to assess an exposure–response relationship. One analysis used the log of TCDD level and a second compared referents to categorical TCDD exposure levels among the workers (< 20 ppt, 20 to < 255 ppt, 255 to < 1,120 ppt, ≥ 1,120 ppt) using dummy variables. We selected the < 20 ppt category as the lowest category because all of the referent serum samples were < 20 ppt. The pregnancies fathered by workers fell into the four exposure categories: 20% of pregnancies had serum values < 20 ppt; 20% of pregnancies had serum values ≥ 1,120 ppt; and the remaining 60% of the pregnancies were split equally into two categories, 20 to < 255 ppt and 255 to < 1,120 ppt. The confounder assessment was carried out for the two main analyses: log of TCDD value at time of conception and categories of TCDD at time of conception.

Because individual pregnancies of the same woman cannot be considered independent events, we conducted analyses using a class of generalized estimating equations that takes into account the correlation of pregnancy outcomes for the same woman, adjusting the odds ratios (ORs) and corresponding standard errors accordingly (*25*). The SAS GENMOD procedure for repeated measures was used to analyze these data.

Sex ratio analysis was limited to live births and included the 15 twin pairs that were excluded from the spontaneous abortion analysis. Logistic regression was used to model the probability of a male birth. We compared the observed sex ratios of worker offspring to referent offspring. Two main analyses characterized each live birth by dioxin level at the time of conception: the log of TCDD level and categorical TCDD levels. We also considered paternal age at first exposure in evaluating the potential effect of TCDD exposure on sex ratio.

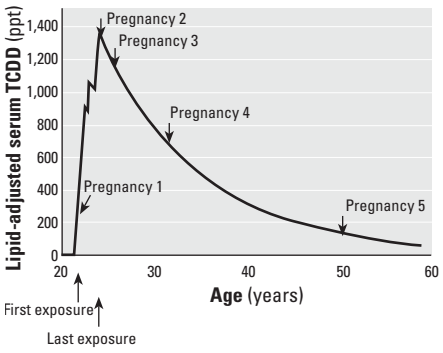

Figure 1. Estimated serum TCDD levels for a worker over time, with exposure periods and five pregnancies indicated.

Confounder assessment was carried out for both analyses for age, education, and race of both mother and father.

Results

Descriptive information on the study participants has been reported previously (*14,15*). A total of 281 workers (70% of the 400 living locatable workers or 48% of the original cohort) participated in the medical examination. To obtain 260 neighborhood referents, it was necessary to invite 938 individuals from the community to participate (28%) (*15*). For this analysis, we included wives of men for whom we had measured or estimated serum TCDD levels, 259 male workers and 243 male referents. Among living current and former wives of these men, we interviewed 245 (77.5%) of the workers' wives and 215 (73.4%) of the referents' wives (Figure 2). As expected, response rate was much higher among current wives (94.2% among wives of workers and 90.5% among wives of referents) than among former wives (45.9% and 29.3% among worker and referent wives, respectively) due to difficulties in locating former wives. To identify potential exposure differences in nonrespondent and respondent wives of workers, we compared the TCDD levels at the time of examination for workers who had at least one participating wife to the TCDD levels of workers for whom no wife participated and found that TCDD levels were similar. Husbands of nonrespondent wives had a mean TCDD level of 213.7 ppt (range, 4.6–1593.4 ppt) in 1987, while husbands of respondent wives had mean TCDD levels of 223.8 ppt (range 2.9–3388.5 ppt).

Among participating women, there were 1,339 eligible pregnancies, 707 pregnancies to referent wives and 632 pregnancies to worker wives. Among pregnancies fathered by the workers, 300 were conceived before exposure at the study company and 332 were conceived during or after exposure. Table 1 provides demographic information on the wives included in the analysis. The women were mostly white, and most had no more than a high school education. The two groups had similar age at first pregnancy. The mean lifetime number of pregnancies and mean number of eligible study pregnancies per woman was also similar for workers and referents.

Table 2 provides descriptive data on the pregnancies included in the analysis. Referents had a higher crude miscarriage rate (12.7%) than did workers. Among pregnancies fathered by workers before exposure, the crude miscarriage rate was only 8.4%. Among pregnancies fathered by workers during or after exposure, the crude miscarriage rate was 10.7%. The same percentage of referent and worker wives worked during their pregnancies. Very few reported work with chemicals or radiation during the pregnancy (between 0.3% and 3.0%). Women with jobs that involved physical stressors were more common (between 8.2% and 12.2%). Both groups of women became pregnant during the same decades. The percentage of women who smoked during pregnancy was similar in both groups; however, wives of workers reported that they smoked more cigarettes per day than did referent wives. Mother's alcohol consumption during pregnancy was lowest (5.0%) for pregnancies fathered by workers before exposure and similar among mothers of referent pregnancies (16.7%) and pregnancies fathered by workers after exposure (19.0%). These differences in smoking and alcohol use were not statistically significant.

The median TCDD value was 6 ppt for referent fathers (range, 2–19 ppt). Paternal TCDD level at time of conception was estimated for all pregnancies fathered by workers during or after exposure at the plant. The median paternal TCDD level at the time of conception was 254 ppt for these pregnancies, ranging from 3 to 16,340 ppt (Table 2).

Many covariates were evaluated for inclusion in the final model, including mother's medication use and medical conditions; mother's education, age, race, and ethnicity; mother's employment and exposure to workplace factors such as chemicals, radiation, and physical stressors, as well as cigarette smoking and alcohol consumption; and whether the father was employed at the Missouri or New Jersey plant. The potential confounders that met retention criteria and were included in the final model were mother's age, mother's Hispanic ethnicity, and thyroid medication during the first trimester (Table 3). The analysis showed no association between paternal TCDD level at

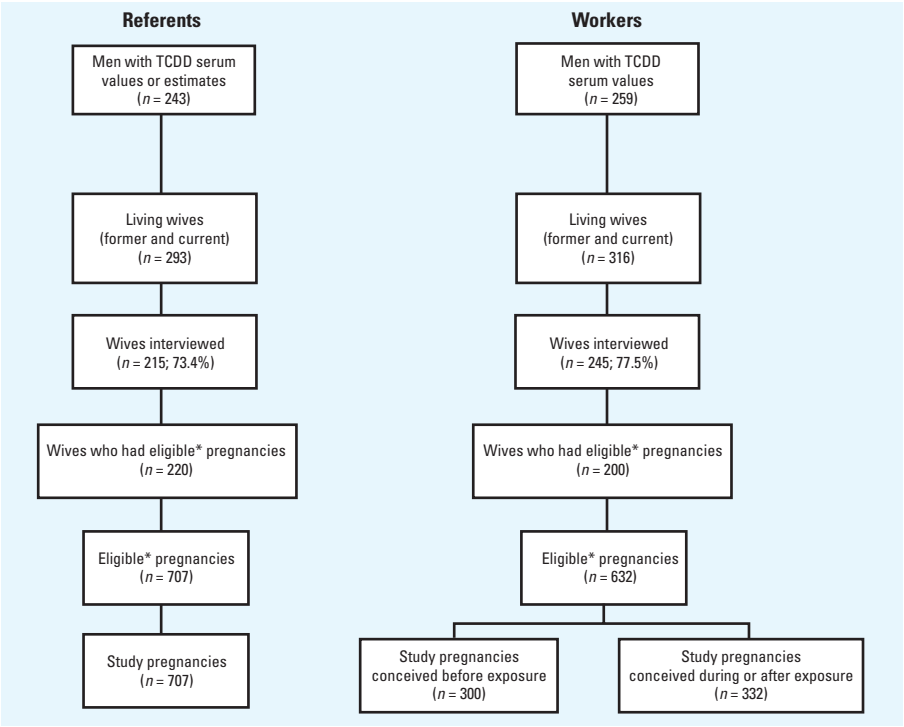

Figure 2. Selection of wives and pregnancies for study.
*Excluded pregnancies: 15 twin sets; 7 tubal pregnancies; 26 induced abortions; 46 pregnancies exposed to contraceptive methods such as birth control pills, intrauterine devices, or injections to induce a menstrual period; 3 Rh factor incompatibility; 1 pregnant at time of interview.

Table 1. Selected characteristics of referent and worker wives.

	Referent wives (*n* = 220)	Worker wives (*n* = 200)
Education: ≤ high school, *n* (%)	136 (61.8)	135 (67.5)
Race: Caucasian, *n* (%)	196 (89.1)	181 (90.5)
Ethnicity: Hispanic, *n* (%)	5 (2.3)	6 (3.0)
Age at interview, years[a]	52.6 ± 10.8 (25.5–74.6)	50.5 ± 10.3 (26.5–75.3)
Age at first pregnancy, years[a]	22.3 ± 4.2 (13.2–38.0)	21.8 ± 4.2 (15.2–38.0)
Lifetime no. of pregnancies[a]	3.7 ± 2.0 (1–11)	3.6 ±1.7 (1–9)
Lifetime no. of live births[a]	3.1 ± 1.7 (0–10)	3.1 ± 1.5 (0–9)
No. of eligible pregnancies[a]	3.2 ± 1.8 (1–11)	3.2 ± 1.7 (1–9)

[a]Mean ± SD (range).

the time of conception and spontaneous abortion when TCDD levels were modeled as a continuous variable [OR = 0.97; 95% confidence interval (CI), 0.88–1.09). When analyzed categorically, no association was observed among pregnancies fathered by workers with TCDD levels of < 20 ppt (OR = 0.77; 95% CI, 0.48–1.22); 20 to < 255 ppt (OR = 0.81; 95% CI, 0.40–1.63); 255 to < 1,120 ppt (OR = 0.69; 95% CI, 0.30–1.58); and ≥ 1,120 ppt (OR = 0.95; 95% CI, 0.42–2.17) compared to pregnancies fathered by referents (Table 3).

When all fetal losses, including the 14 stillbirths that occurred after 20 weeks of gestation, were included in the continuous model, the odds ratio was unchanged (0.97). Separate analyses of the 85 early (≤ 8 weeks) and 77 late (> 8 weeks) fetal losses showed similar results (OR = 0.99 and 0.97, respectively).

Because there were some differences between the wives of workers and referents, including a somewhat lower spontaneous abortion rate among workers' wives before exposure (Table 2), we conducted an analysis using the final model but limited to pregnancies fathered by workers during or after exposure. This analysis also failed to show a statistically significant dose–response relationship, but it did show a slightly increased odds ratio in the uppermost TCDD category (OR = 1.36; 95% CI, 0.39–4.81; Table 4).

Some wives in this population could have been exposed directly to TCDD if their husbands brought TCDD-contaminated clothing home while employed at the plant (take-home exposure). We do not have data on the potential for take-home exposures to TCDD. However, those pregnancies that overlapped the fathers' dates of exposure at the plant would be expected to have the highest potential for take-home exposure. To examine this possibility, we identified those pregnancies whose first trimester fell completely within the fathers' dates of exposure at the plant. Using the final model, we compared the risk of spontaneous abortion among these 78 pregnancies to pregnancies fathered by workers before exposure and pregnancies fathered by referents before or during the period that the plants operated. Overall, no increased risk for spontaneous abortion was observed (OR = 0.84; Table 5). Paternal TCDD level at conception is a measure of cumulative TCDD exposure, but serum TCDD level could also reflect a greater potential for bringing contamination home and thus could be used as a surrogate for take-home exposure. The 27 pregnancies with both potential for take-home exposure and paternal TCDD ≥ 1,120 ppt had an increased odds ratio with wide confidence intervals (OR = 1.38; 95% CI, 0.46–4.18; Table 5).

There were 1,191 live births (544 fathered by workers and 647 fathered by referents). Sex ratio did not differ by TCDD exposure (0.53 for all worker births and 0.54 for referent births) or by paternal TCDD serum level (Table 6). In regression analyses, only father's race and mother's education met the criteria for inclusion as potential confounders. This analysis did not show a trend of lower probability of a male birth with increasing serum TCDD level. The sex ratio for the 17% of workers who were first exposed to TCDD before age 20 was not different from those who were first exposed at age 20 or older (0.59, 95% CI, 0.53–0.66 and 0.56, 95% CI, 0.53–0.59, respectively). When TCDD level at time of conception and age at first exposure were included in the same model, no change was observed in the adjusted odds ratios: < 20 ppt (OR = 1.00); 20 to < 255 ppt (OR = 0.90; 95% CI, 0.43–1.86); 255 to < 1,120 ppt (OR = 0.81; 95% CI, 0.39–1.71); and ≥ 1,120 ppt (OR = 0.98; 95% CI, 0.43–2.24).

Discussion

TCDD exposure levels in this cohort were substantially higher than levels reported in most studies that have been conducted (*24*). When extrapolated back to time of conception, the serum TCDD levels among the workers ranged from 3 to 16,340 ppt, with a median level of 2,167 ppt in the highest exposure group. The Ranch Hand cohort also extrapolated serum TCDD levels back to the time of conception and estimated a range of 0–1,424 ppt with a median TCDD level of 153 ppt in the highest exposure group (*9*). Overall, our analysis did not indicate a trend toward increased risk with

Table 2. Selected characteristics of pregnancies to wives of workers and referents.

Characteristic	Pregnancies fathered by: Referents (n = 707)[a]	Workers before exposure (n = 300)	Workers during or after exposure (n = 332)[a]
No. of miscarriages (≤ 20 weeks)	89 (12.7)	25 (8.4)	35 (10.7)
No. of stillbirths (> 20 weeks)	6 (0.7)	3 (0.9)	4 (1.0)
No. of live births	612 (86.6)	272 (90.7)	293 (88.3)
Mother's work during pregnancy			
No. worked during pregnancy	204 (28.2)	86 (29.0)	94 (28.4)
No. worked with chemicals	15 (2.1)	9 (3.0)	2 (0.6)
No. worked with radiation	3 (0.4)	1 (0.3)	2 (0.6)
No. work involved heavy lifting, continuous standing, or use of vibrating tools	85 (12.2)	30 (10.1)	27 (8.2)
Alcohol consumption:			
> 2 drinks/week in pregnancy	116 (16.7)	15 (5.0)	63 (19.0)
Cigarette smoking			
Ever in first month of pregnancy	179 (25.3)	81 (27.0)	99 (29.8)
First trimester			
< 20 cigarettes/day	100 (14.1)	36 (12.0)	36 (10.8)
> 20 cigarettes/day	76 (10.8)	42 (14.0)	57 (17.2)
Mean year of conception (range)	1960 (1935–1987)	1956 (1935–1971)	1966 (1951–1987)
Mother's age at conception (range)	26.3 (13.2–44.0)	23.4 (15.3–38.0)	27.5 (16.1–43.1)
Median paternal TCDD ppt at conception (range)	6 (2–19)	6[b]	254 (3–16,340)

Values shown are number (%) except where indicated.
[a]Percentages may not be of entire group due to missing information for some fields. [b]All before exposure conceptions to workers were assigned the median referent value of 6 ppt.

Table 3. Spontaneous abortion: adjusted ORs for paternal TCDD serum level and other variables.

Variable	No. of spontaneous abortions/ no. of pregnancies	OR (95% CI)
Maternal age (years)	—	1.06 (1.02–1.09)
Hispanic ethnicity		
No	137/1,283	1.00
Yes	6/34	2.30 (0.90–5.89)
Thyroid disease medication		
No	138/1,300	1.00
Yes	5/17	4.04 (1.77–9.16)
Pregnancies by log(TCDD level)	143/1,317	0.97 (0.88–1.09)
Paternal TCDD level at conception		
Referent pregnancies (< 20 ppt)	84/696	1.00
Worker pregnancies		
< 20 ppt	29/344	0.77 (0.48–1.22)
20 to < 255 ppt	11/113	0.81 (0.40–1.63)
255 to < 1,120 ppt	11/98	0.69 (0.30–1.58)
≥ 1,120 ppt	8/66	0.95 (0.42–2.17)

increasing TCDD level. Separate analyses of all fetal loss and early or late fetal loss were not associated with paternal TCDD serum level. Both the exposed pregnancies and the unexposed pregnancies had miscarriage rates that were within the expected range.

We examined other possible explanations for our findings. Although our response rate was acceptable (73.4% and 77.5% among referents' and workers' wives, respectively), it is possible that this group was not representative because we only contacted wives of men who had already agreed to participate in the cross-sectional medical study.

The spontaneous abortion rate among the workers' wives before exposure was somewhat lower compared to the rates among the wives of workers after exposure. This could be due, in part, to younger maternal age during the pre-employment period. However, we did not observe a similar pattern among referent wives. The spontaneous abortion rate among referents' wives in the years before the date of plant operation was not different from the rate in the years after the plants began operation (12.8% vs. 12.5%, respectively). The women reported information on spontaneous abortion, and we were not able to verify the reports with medical records. Thus, there is a possibility of differences in recall between the groups. However, it seems unlikely that the exposed population would have poorer recall than the referent group. It is possible that the groups of wives differ in ways that we have not been able to identify by analysis of demographics. When we restricted analysis to those pregnancies fathered by workers during or after exposure, we found higher point estimates than we found in the unrestricted analysis at each TCDD level with a slightly increased odds ratio in the highest TCDD category. This provides some suggestion that the groups differed in ways that we could not measure.

The pregnancies in this study occurred primarily during the 1950s and 1960s, before early pregnancy tests were available. Thus, this investigation focused on collection of data regarding the outcomes of recognized clinical pregnancies. We could not examine whether paternal TCDD serum level was related to early (subclinical) fetal loss.

Some workers' wives also had the potential for direct exposure via take-home contamination from the plant. We had no measure of take-home contamination, but analyses of pregnancies whose first trimester fell within the time of father's exposure at the plant did not indicate an increased risk for spontaneous abortion. Pregnancies with both potential take-home exposure and high paternal serum TCDD levels (≥ 1,120 ppt) had the highest odds ratio, but this estimate is based on a small number of spontaneous abortions ($n = 4$).

In summary, we did not find an association between paternal serum TCDD level and spontaneous abortion in this population. The findings are similar to previous epidemiologic studies of paternal TCDD exposure and fetal loss. Because the estimated TCDD levels in this population were much higher than other studies, the results provide additional evidence that paternal TCDD exposure does not increase the risk of spontaneous abortion at levels above those observed in the general population.

It has been suggested that exposure to endocrine-disrupting chemicals may lower the percentage of male births in exposed populations. The findings in this study indicate that sex ratio among offspring is not markedly affected among adults exposed to high levels of TCDD. Although these men were exposed to levels hundreds of times higher than the general population, their exposure occurred during adulthood. The study could not evaluate the effect of father's childhood or prenatal TCDD exposure on subsequent sex ratio.

Suggestive evidence of a relationship between dioxin exposure and some central nervous system birth defects has been observed in other studies (*8,26,27*). Analyses of major birth defects and other adverse pregnancy outcomes, including birth weight, preterm birth, and reduced fertility in this population are under way.

Table 4. Spontaneous abortion: adjusted ORs for paternal TCDD serum level, pregnancies fathered by workers during or after exposure.

Variable	No. of spontaneous abortions/ no. of pregnancies	OR[a] (95% CI)
Pregnancies by log(TCDD level)	35/328	1.04 (0.85–1.27)
Paternal TCDD level at conception		
< 20 ppt	5/51	1.00
20 to < 255 ppt	11/113	1.05 (0.33–3.42)
255 to < 1,120 ppt	11/98	0.88 (0.25–3.10)
≥ 1,120 ppt	8/66	1.36 (0.39–4.81)

[a]Adjusted for maternal age, Hispanic ethnicity, and thyroid disease medication.

Table 5. Spontaneous abortion: adjusted ORs for potential take-home exposure to TCDD.

Variable	No. of spontaneous abortions/ no. of pregnancies	OR[a] (95% CI)
Unexposed pregnancies[b]	92/865	1.00
Pregnancies with first trimester falling completely within father's exposure dates	8/78	0.84 (0.36-2.11)
Pregnancies by log(TCDD level)	100/943	0.96 (0.80–1.15)
Unexposed pregnancies	92/865	1.00
Pregnancies with first trimester falling within father's dates of exposure by paternal TCDD level at conception		
< 255 ppt	2/28	0.74 (0.17–3.32)
255 to < 1,120 ppt	2/23	0.47 (0.10–2.12)
≥ 1,120 ppt	4/27	1.38 (0.46–4.18)

[a]Adjusted for maternal age, Hispanic ethnicity and thyroid disease medication. [b]Unexposed pregnancies fathered by workers that ended before exposure and referent pregnancies fathered before or during the TCDD exposure period at the plants.

Table 6. Sex ratio and adjusted ORs by paternal serum TCDD level.

Variable	No. of children			Sex ratio (95% CI)	OR[a] (95% CI)
	Males	Females	Total		
Referent pregnancies (< 20 ppt)	352	295	647	0.54 (0.52–0.56)	1.00
Worker pregnancies	290	254	544	0.53 (0.51–0.55)	1.08 (0.85–1.38)
< 20 ppt	148	144	292	0.51 (0.48–0.54)	1.22 (0.93–1.60)
20 to < 255 ppt	59	45	104	0.57 (0.52–0.62)	0.92 (0.59–1.44)
255 to < 1,120 ppt	50	38	88	0.57 (0.52–0.62)	0.89 (0.52–1.46)
≥ 1,120 ppt	33	27	60	0.55 (0.49–0.61)	1.03 (0.55–1.01)
Pregnancies by log(TCDD level)					0.96 (0.90–1.03)

[a]Adjusted for mother's education and father's race.

References and Notes

1. Institute of Medicine. Veterans and Agent Orange: Update 1996. Washington, DC:National Academy Press, 1996.
2. U.S. EPA. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds. Final Draft. EPA/600/00/001. Washington, DC:U.S. Environmental Protection Agency, 2000.
3. Smith AH, Fisher DO, Pearce N, Chapman CJ. Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Arch Environ Health 37:197–200 (1982).
4. Suskind RR, Hertzberg VS. Human health effects of 2,4,5-T and its toxic contaminants. JAMA 251:2372–2380 (1984).
5. Townsend JC, Bodner KM, Van Peenen PFD, Olson RD, Cook RR. Survey of reproductive events of wives of employees exposed to chlorinated dioxin. Am J Epidemiol 115:695–713 (1982).
6. Aschengrau A, Monson RR. Paternal military service in

Vietnam and risk of spontaneous abortion. J Occup Med 31:618–623 (1989).
7. Stellman SD, Stellman JM, Sommer JF Jr. Health and reproductive outcomes among American Legionnaires in relation to combat and herbicide exposure in Vietnam. Environ Res 472:150–174 (1988).
8. Wolfe WH, Michalek JE, Miner JC, Rahe AJ, Moore CA, Needham LL, Patterson DG Jr. Paternal serum dioxin and reproductive outcomes among veterans of Operation Ranch Hand. Epidemiology 6:17–22 (1995).
9. Michalek JE, Rahe AJ, Boyle CA. Paternal dioxin, preterm birth, intrauterine growth retardation and infant death. Epidemiology 9(2):161–167 (1998).
10. Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr, Needham LL. Change in sex ratio with exposure to dioxin [Letter]. Lancet 353:409 (1996).
11. Mocarrelli P, Gerthoux PM, Ferrari E, Patterson DG Jr, Kieszak SM, Brambilla P, Vincoli N, Signorini S, Tramacere P, Carreri V, et al. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355:1858–1863 (2000).
12. Michalek JE, Rahe AJ, Boyle CA. Paternal dioxin and the sex of children fathered by veterans of Operation Ranch Hand [Letter]. Epidemiology 9:474–475 (1998).
13. Egeland GM, Sweeney MH, Fingerhut MA, Wille KK, Schnorr TM, Halperin WE. Total serum testosterone and gonadotropins in workers exposed to dioxin. Am J Epidemiol 139(3):272–281 (1994).
14. Sweeney MH, Fingerhut MA, Connally LB, Halperin WE, Moody PL, Marlow DA. Progress of the NIOSH cross-sectional medical study of workers occupationally exposed to chemicals contaminated with 2,3,7,8-TCDD. Chemosphere 19:973–977 (1989).
15. Sweeney MH, Fingerhut MA, Arezzo JC, Hornung RW, Connally LB. Peripheral neuropathy after occupational exposure to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD). Am J Ind Med 23:845–858 (1993).
16. Fingerhut MA, Sweeney MH, Patterson DG, Piacitelli LA, Morris JA, Marlow DA, Hornung RW, Cameron LW, Connally LB, Needham LL, et al. Levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the serum of US chemical workers exposed to dioxin contaminated products: interim results. Chemosphere 19:835–840 (1989).
17. Patterson DG, Holler JS, Lapeza CR Jr, Alexander LR, Groce DF, O'Connor RC, Smith SJ, Liddle JA, Needham LL. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human adipose tissue for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Anal Chem 58:705–713 (1986).
18. Patterson DG, Fingerhut MA, Roberts DW, Needham LL, Sweeney MH, Marlow DA, Andrews JS Jr, Halperin WE. Levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Am J Ind Med 16:135–146 (1989).
19. Turner WE, Isaacs S, Alexander LR, Patterson DG. A quality assurance program for large-scale studies measuring 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human serum. Chemosphere 18:1009–1016 (1989).
20. Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. N Engl Med 324:212–218 (1991).
21. Piacitelli LA, Haring Sweeney M, Fingerhut MA, Patterson DG Jr, Turner WE, Connally LB, Wille KK, Tompkins B. Serum levels of PCDDs and PCDFs among workers exposed to 2,3,7,8-TCDD contaminated chemicals. Chemosphere 25:251–254 (1992).
22. Dankovic DA, Andersen ME, Salvan A, Stayner LT. A simplified PBPK model describing the kinetics of TCDD in humans [Abstract]. Toxicologist 15:272 (1995).
23. Thomaseth K, Salvan A. Estimation of occupational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using a minimal physiologic toxicokinetic model. Environ Health Perspect 106(suppl 2):743–753 (1998).
24. Haring Sweeney M, Fingerhut MA, Patterson DG, Connally LB, Piacitelli LA, Morris JA, Greife AL, Hornung RW, Marlow DA, Dugle JE, et al. Comparison of serum levels of 2,3,7,8-TCDD in TCP production workers and in an unexposed comparison group. Chemosphere 20:993–1000 (1990).
25. Zeger SL, Liang K-Y. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42:121–130 (1986).
26. CDC. Health Status of Vietnam Veterans. Vietnam Experience Study. Vols I-V, Supplements A-C. Atlanta, GA:Centers for Disease Control, 1989.
27. Erickson JD, Mulinare J, McClain PW. Vietnam veteran's risks for fathering babies with birth defects. JAMA 252:903–912 (1984).