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Chevrier, Jonathan
Warner, Marcella
Gunier, Robert B
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Serum Dioxin Concentrations and Thyroid Hormone Levels in the Seveso Women's Health Study

Jonathan Chevrier*, Marcella Warner, Robert B. Gunier, Paolo Brambilla, Brenda Eskenazi, and Paolo Mocarelli

* Correspondence to Dr. Jonathan Chevrier, Department of Epidemiology, Biostatistics and Occupational Health, Faculty of Medicine, McGill University, 1020 avenue des Pins ouest, Montréal, Québec H3A 1A2, Canada (e-mail: jonathan.chevrier@mcgill.ca).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent environmental contaminant. Although experimental evidence suggests that TCDD alters thyroid hormone levels in rodents, human data are inconsistent. In 1976, a trichlorophenol plant exploded in Seveso, Italy. Women living in highly exposed areas were followed through the Seveso Women's Health Study. TCDD concentrations were measured in 1976 ($n = 981$) and 1996 ($n = 260$), and levels of total thyroxine, free thyroxine, free triiodothyronine, and thyroid-stimulating hormone were measured in 1996 ($n = 909$) and 2008 ($n = 724$). We used conditional multiple linear regression and marginal structural models with inverse-probability-of-treatment weights to evaluate associations and causal effects. TCDD concentration in 1976 was inversely associated with total thyroxine level in 1996 but not in 2008. Associations were stronger among women who had been exposed before menarche. Among these women, associations between total thyroxine and concurrent 1996 TCDD were slightly weaker than those with 1976 TCDD. A model including both 1976 and 1996 measurements strengthened the relationship between 1976 TCDD and total thyroxine but drove the association with 1996 TCDD to the null. TCDD exposure was not associated with levels of other thyroid hormones. TCDD exposure, particularly exposure before menarche, may have enduring impacts on women's total thyroxine levels. Initial exposure appears to be more influential than remaining body burden.

accidents; dioxin; endocrine disruptors; environmental exposure; TCDD; thyroid hormones

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a persistent lipophilic environmental contaminant produced as an unwanted by-product of various combustion and chemical processes (1), is a known human carcinogen and endocrine disruptor (2, 3). Experimental animal data suggest that TCDD causes decreases in total and free thyroxine (T_4) levels, with some studies suggesting increases in thyroid-stimulating hormone (TSH) levels (4–6).

Only 4 human studies have investigated associations between serum TCDD concentrations and thyroid hormone levels in adults, and results have been inconsistent. Ott et al. (7) reported positive associations between T_4 and whole-blood TCDD concentrations measured in 131 trichlorophenol production plant workers more than 45 years after an acci-

dental exposure to TCDD. Chloracne status and TCDD levels estimated by back-extrapolation to the time of the accident were also positively associated with T_4 levels. There were no associations with TSH. Similarly, Calvert et al. (8) found that high serum TCDD concentrations ($\geq 1,860$ pg/g lipids) measured more than 15 years after the last exposure were associated with an elevated free T_4 index among 278 trichlorophenol plant workers relative to 257 referents with lower exposure (mean serum TCDD concentration = 7 pg/g lipids), although no clear exposure-response relationship was demonstrated. No association was found between TCDD and total T_4 or TSH. In 32 Australian herbicide spray workers, back-extrapolated serum TCDD concentrations (but not concurrent levels) were negatively correlated with triiodothyronine (T_3)

but not T_4 (9). Finally, in a large ($n = 1,009$ exposed and 1,429 referents) prospective study of US Air Force veterans who sprayed TCDD-contaminated Agent Orange in Vietnam between 1962 and 1971, Pavuk et al. (10) reported positive associations between serum TCDD concentrations in 1982–1997 and concomitantly measured TSH levels (but not total T_4 levels, free T_4 index, or T_3% uptake). Except for the study by Calvert et al. (8), which included only 31 women, these studies were exclusively conducted among males.

Although few data are available on TCDD's potential for thyroid hormone disruption in women, a number of studies of other polychlorinated dibenzodioxins, dibenzofurans, and biphenyls whose dioxin-like activity was evaluated using toxic equivalents (TEQs) have been conducted, with similarly inconsistent results (11–18).

On July 10, 1976, an explosion at a trichlorophenol manufacturing plant in Seveso, Italy, released approximately 30 kg of TCDD over the surrounding area, resulting in the highest known exposure to TCDD in a residential population (19). The Seveso Women's Health Study, initiated in 1996, is a historical cohort study of the female population residing around Seveso at the time of the explosion (20). As part of the Seveso Women's Health Study, TCDD was measured in blood samples collected shortly after the accident, creating a unique opportunity to study the long-term health effects of TCDD exposure with limited potential for reverse causality, a bias that may have affected prior studies. Here, we examined the relationship between 1976 serum TCDD levels and thyroid hormone concentrations measured in 1996 and 2008. In a subset, we also examined the relationship between 1996 serum TCDD/TEQ levels and thyroid hormone concentrations measured in 1996 and 2008.

METHODS

Participants

Details on the design of the Seveso Women's Health Study are presented elsewhere (20). Women who were aged 40 years or less in 1976, resided in the most highly contaminated areas (zone A or B, based on surface soil measurements (21)) at the time of the explosion, and had sufficient serum collected soon after the explosion were eligible to participate in the study. Enrollment in the Seveso Women's Health Study cohort took place between March 1996 and July 1998 (referred to hereafter as "the 1996 visit"), and 981 women (80% of those eligible) participated. The cohort was followed up between April 2008 and December 2009 (referred to hereafter as "the 2008 visit"); of those women who were still alive and could be located ($n = 929$), 833 (90%) participated in this second wave.

To study associations between 1976 TCDD concentrations and 1996 thyroid hormone levels, we excluded women who did not provide a blood sample in 1996 ($n = 25$), had a history of thyroid disease before the explosion ($n = 20$), had Turner's syndrome ($n = 1$), or reported current treatment for thyroid disease ($n = 26$), leaving a final sample of 909. For the analysis of 1996 TCDD/TEQ and 1996 thyroid hormone levels, the sample was further limited to those with 1996 serum TCDD/TEQ measurements ($n = 260$).

To evaluate associations between 1976 TCDD and 2008 thyroid hormone levels, we excluded women who did not provide a blood sample in 2008 ($n = 27$), had a history of thyroid disease before the explosion ($n = 16$) or reported treatment for thyroid disease in 2008 ($n = 61$), had Turner's syndrome ($n = 1$), or were pregnant ($n = 4$), leaving a final sample of 724. For the analysis of 1996 TCDD/TEQ and 2008 thyroid hormone levels, the sample was further limited to those with 1996 serum TCDD/TEQ measurements ($n = 237$).

This study was approved by the University of California, Berkeley, Committee for the Protection of Human Subjects, and written informed consent was obtained from all women before participation.

Procedure

At both the 1996 and 2008 study visits, women underwent a fasting blood draw, anthropometric measurements, and a structured personal interview by a nurse who was blinded to zone of residence and to TCDD and serum thyroid hormone concentrations. Information collected included demographic and lifestyle characteristics and reproductive and medical histories.

Measurement of TCDD

We measured TCDD in archived sera collected soon after the explosion by high-resolution gas chromatography/high-resolution mass spectrometry; coefficients of variation were 13%–15% (22). Details of serum sample selection and TCDD concentrations are presented elsewhere (20, 23). Values are reported on a lipid-weight basis in parts per trillion (ppt) (24). For values below the limit of detection (9.4% in 1976 and 7.7% in 1996), a serum TCDD level of one-half the detection limit was assigned. The median serum sample weight was 0.65 g, and the median limit of detection was 18.8 ppt, lipid-adjusted. TEQ was not assessed in the 1976 samples because of limited sample volume.

For a subset of participants ($n = 260$), we measured levels of 17 polychlorinated dibenzodioxins/dibenzofurans, including TCDD, 4 coplanar polychlorinated biphenyls, and 8 mono-*ortho*-substituted polychlorinated biphenyls, in serum samples collected during the 1996 study by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (25, 26). Total TEQ concentration was calculated on the basis of the 2005 WHO Toxicity Equivalency Factors (27). Details on TCDD and total TEQ concentrations in 1996 serum are presented elsewhere (28).

Measurement of thyroid hormone levels

Thyroid hormone levels were measured at the Hospital of Desio laboratory in serum samples collected in 1996 and 2008 using sandwich (TSH) or competitive (free T_3, free T_4, and total T_4) electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany). The limits of detection for TSH, free T_3, free T_4, and total T_4 were 0.01 mIU/L, 0.04 ng/dL, 0.02 ng/dL, and 0.4 μg/dL, respectively. Coefficients of variation ranged between 1.5% and 6.2%.

Statistical analysis

Serum TCDD concentrations were heavily right-skewed, with a wide distribution, and thus the data were $\log_{10}$-transformed to reduce the influence of outliers. We also $\log_{10}$-transformed TSH values in order to normalize residuals. Free T_3, free T_4, and total T_4 values were approximately normally distributed and were thus expressed on the arithmetic scale. We used analysis of variance to compare serum TCDD concentrations across demographic categories and Pearson's correlations to assess bivariate associations of continuous variables. We used multiple linear regression to evaluate the relationship between serum TCDD concentrations and thyroid hormone levels. We first fitted generalized additive models with a 3–degrees of freedom cubic spline to evaluate the shape of exposure-response curves. None of the digression from linearity tests was significant when a liberal P value ($P < 0.15$) was used. We therefore expressed TCDD linearly (on the $\log_{10}$ scale) in regression models. Regression coefficients represent the mean (free T_3, free T_4, and total T_4) or percent (TSH) change for each 10-fold increase in serum TCDD concentration.

We fitted separate models for associations between TCDD and thyroid hormone levels at the different time points. In order to identify the conditionally independent associations of 1976 and 1996 TCDD with thyroid hormone levels, we also fitted models including TCDD measures at both time points. In addition, we used multiple logistic regression to evaluate whether exposure was associated with increased odds of hypothyroidism or hyperthyroidism, based on reference ranges provided by the laboratory or physician diagnosis. Women who had TSH levels greater than 4.5 mIU/L, had free T_4 levels less than 0.9 ng/dL, or were taking thyroid hormone supplements were classified as hypothyroidic ($n = 101$ in 1996 and $n = 111$ in 2008); those who had TSH levels less than 0.1 mIU/L, had free T_4 levels greater than 1.9 ng/dL, or were being treated for hyperthyroidism were considered hyperthyroidic ($n = 31$ in 1996 and $n = 30$ in 2008).

Based on the current literature and directed acyclic graphs, we considered the following variables as potential confounders: age, education, marital status, and history of cigarette smoking and alcohol consumption at the time of thyroid hormone measurement. We included in the models covariates that were associated with both thyroid hormone levels and TCDD/TEQ at $P < 0.2$ in bivariate analyses. Final models included age at thyroid hormone measurement and age at thyroid hormone measurement squared. We considered effect modification by age and menarcheal status at the time of the explosion based on previous results in this population by including cross-product terms in the models and performing Wald tests (29, 30).

In sensitivity analyses, we refitted the models after excluding outliers with studentized residuals greater than 3. Excluding 2 outliers with suppressed TSH concentrations (<0.01 mIU/L) substantially altered results for the associations with 2008 TSH levels, so we report results for this model without them. To investigate whether the lipid adjustment method that we chose affected our results, we refitted the 1996 TCDD models with wet-weight TCDD (ng/g serum) concentrations, including total lipids as a covariate. In an attempt to adjust for selection bias due to loss to follow-up, we applied stabilized inverse probability weights to the regression models (31). We determined weights using multiple logistic regression with independent variables selected using a "Super Learner" algorithm with V-fold cross-validation (32). To examine the possibility of reverse causality, we also repeated analyses after restricting the data to the 724 participants for whom serum samples had been collected within 90 days of the explosion. Lastly, we reran the analyses with values below the limits of detection randomly imputed based on a log-normal probability distribution whose parameters were determined by maximum likelihood estimation (33).

We repeated the above analyses to estimate causal effects using marginal structural models (34) for continuous exposure following the procedure used by Cerda et al. (35). Inverse-probability-of-treatment weights were determined on the basis of probability density functions whose parameters were estimated using linear regression with TCDD as the dependent variable. To improve the precision of estimates, we used stabilized weights equal to the marginal probability densities divided by probability densities conditional on covariates measured at or before the time of thyroid hormone measurements. When assessing effect modification, both the numerator and the denominator of the weights were conditioned on the effect modifier to further improve precision (34). Conditional probability densities were determined by using the "Super Learner" prediction algorithm described above (32). Weights greater than 10 were truncated at this value. Note that marginal structural and conditional models estimate different quantities. Estimates from these methods may not be directly compared, but conclusions reached using each method may be contrasted. Statistical significance was set at $P < 0.05$ for main effects and $P < 0.10$ for effect modification using 2-tailed tests. Analyses were conducted using STATA, version 11.2 (StataCorp LP, College Station, Texas) and R, version 3.15.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Population

The average age of participants at the time of the explosion was 19.7 years (standard deviation (SD), 11.2). In 1996, participants had a mean age of 40.3 years (SD, 11.5), 46% had completed high school, and 30% were overweight or obese (Table 1). Most never consumed alcohol or smoked cigarettes (64% and 65%, respectively), and 52% were multiparous. In 2008, the average age of participants was 51.6 years (SD, 11.0); 55% were overweight or obese; 64% and 63%, respectively, abstained from alcohol and cigarettes; and 65% were multiparous.

TCDD/TEQ serum concentrations

Median serum TCDD concentrations were 60.2 ppt (interquartile range, 28.5–163.0) and 7.0 ppt (interquartile range, 4.2–12.9) in 1976 and 1996, respectively, and the measures were moderately correlated ($r = 0.59$, $P < 0.001$). The median total TEQ in 1996 was 25.6 ppt (interquartile range,

Table 1. Demographic Characteristics of Participants at the Time of TCDD Measurement, Seveso Women's Health Study, Italy, 1976–2009

Characteristic	1976 (*n* = 909)		1996 (*n* = 260)	
	No.	%	No.	%
At Time of Explosion (1976)				
Age, years				
0–10	223	24.5	131	50.4
11–20	268	29.5	117	45.0
21–30	220	24.2	10	3.9
31–40	198	21.8	2	0.8
Menarcheal status				
Premenarche	272	29.9	161	61.9
Postmenarche	637	70.1	99	38.1
At Time of 1996 Interview				
Age, years				
20–30	207	22.8	121	46.5
31–40	272	29.9	125	48.1
41–50	223	24.5	12	4.6
51–62	207	22.8	2	0.8
Parity				
0	253	27.8	108	41.5
1	184	20.2	82	31.5
≥2	472	51.9	70	26.9
Educational level				
Less than high school	494	54.4	67	25.8
High school	372	40.9	172	66.2
More than high school	43	4.7	21	8.0
Body mass index[a] category				
<18.5	52	5.7	25	9.6
18.5–24.9	582	64.0	192	73.9
25.0–29.9	202	22.2	31	11.9
≥30.0	73	8.0	12	4.6
Alcohol consumption				
Never drinker	580	63.8	186	71.5
Current drinker				
<1 drink/day	194	21.3	56	21.5
≥1 drink/day	135	14.9	18	7.0
Cigarette smoking				
Never smoker	592	65.1	157	60.4
Former smoker	128	14.1	44	16.9
Current smoker				
≤10 cigarettes/day	119	13.1	44	16.9
>10 cigarettes/day	70	7.7	15	5.8
Marital status				
Never married	173	19.0	78	30.0
Ever married	736	81.0	182	70.0

Abbreviation: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
[a] Weight (kg)/height (m)^2.

19.7–35.8). As previously reported (23), 1976 TCDD concentrations were inversely correlated with age at the time of the explosion ($r = -0.25$, $P < 0.001$) and were substantially higher among participants who were aged 10 years or less (median, 165.0 ppt) than among those aged more than 10 years (median, 46.5 ppt). Likewise, 1976 TCDD concentrations were higher among women who were premenarcheal at the time of the explosion and women who were nulliparous or had never been married in 1996, all of which are age-related (23). TCDD levels in 1976 and 1996 were substantially elevated relative to females of similar ages with background exposure, though levels of other dioxin-like chemicals were not (28).

Thyroid hormone concentrations

Mean free T_3, free T_4, and total T_4 concentrations were 0.4 ng/dL (SD, 0.1), 1.2 ng/dL (SD, 0.5), and 8.1 μg/dL (SD, 1.9) in 1996 and 0.3 ng/dL (SD, 0.1), 1.2 ng/dL (SD, 0.2), and 8.2 μg/dL (SD, 1.7) in 2008, respectively. Geometric mean values for TSH were 1.9 mIU/L (geometric SD, 1.3) in 1996 and 1.8 mIU/L (geometric SD, 2.4) in 2008. Age was inversely associated with free T_4 ($r = -0.17$, $P < 0.001$), total T_4 ($r = -0.11$, $P < 0.01$), and TSH ($r = -0.10$, $P < 0.01$) levels in 1996 but associated only with TSH level ($r = -0.07$, $P = 0.05$) in 2008. No associations were observed between covariates and free T_3 at either time point. Women who were postmenarcheal at the time of the explosion or who were married or multiparous in 1996 had lower serum concentrations of free T_4, total T_4, and TSH (see Web Table 1, available at http://aje.oxfordjournals.org/). Education was positively associated with free T_4 and appeared to have an inverse U-shaped relationship with TSH. Alcohol consumption was inversely associated with total T_4, while cigarette smoking was positively related to free T_4.

Associations between TCDD and thyroid hormone levels

We found that 1976 TCDD was inversely associated with total T_4 level in 1996 ($\beta = -0.27$, 95% confidence interval (CI): −0.49, −0.05) (Table 2). The association was stronger among participants who were premenarche at the time of the explosion ($\beta = -0.63$, 95% CI: −1.11, −0.15) relative to those who were postmenarche ($\beta = -0.13$, 95% CI: −0.37, 0.11; P for interaction = 0.05). We found a weak, nonsignificant association between 1976 TCDD and 2008 total T_4 levels ($\beta = -0.11$, 95% CI: −0.32, 0.12) and little evidence of effect modification by menarcheal status at explosion. Including 1976 and 1996 TCDD levels in the same model (Figure 1) resulted in stronger associations between 1976 TCDD and 1996 total T_4 overall ($\beta = -0.42$, 95% CI: −0.96, 0.12) and for women who were premenarcheal at the time of the explosion ($\beta = -1.09$, 95% CI: −1.77, −0.41), but it drove the associations with 1996 TCDD towards the null ($\beta = -0.04$, 95% CI: −0.72, 0.64).

There was no clear evidence of a relationship between 1976 TCDD and TSH, free T_4, or free T_3 measured in 1996 or 2008. We also found no clear evidence of associations between 1996 TCDD and any of the thyroid hormones in 1996 or 2008, regardless of menarcheal status at explosion (Table 3). Although we did observe a weak inverse association between 1996

Table 2. Associations Between 1976 Log$_{10}$ TCDD Concentration (Parts per Trillion) and Thyroid Hormone Levels by Menarcheal Status at the Time of the Explosion (1976), Seveso Women's Health Study, Italy, 1976–2009

Thyroid Hormone	No. of Participants[a]	Adjusted[b] β[c]	95% CI	Menarcheal Status at Explosion						P for Interaction
				Premenarche			Postmenarche			
				No.	Adjusted[b] β[c]	95% CI	No.	Adjusted[b] β[c]	95% CI	
1996 Thyroid Hormone Measurements										
TSH[d]	909	6.61	−0.87, 14.66	272	0.38	−9.34, 11.13	637	9.27	−0.76, 20.32	0.35
Total T$_4$	889	−0.27	−0.49, −0.05	260	−0.63	−1.11, −0.15	629	−0.13	−0.37, 0.11	0.05
Free T$_4$	894	0.01	−0.04, 0.06	260	0.02	−0.07, 0.12	634	0.01	−0.05, 0.07	0.72
Free T$_3$	895	0.00	−0.02, 0.01	260	0.00	−0.02, 0.02	635	−0.01	−0.03, 0.02	0.70
2008 Thyroid Hormone Measurements										
TSH[d]	722	5.44	−3.39, 15.08	225	4.82	−8.69, 20.33	497	6.41	−4.94, 19.10	0.42
Total T$_4$	722	−0.11	−0.32, 0.12	225	0.09	−0.31, 0.48	497	−0.24	−0.48, 0.00	0.13
Free T$_4$	724	0.00	−0.02, 0.03	225	0.01	−0.03, 0.05	499	0.00	−0.03, 0.03	0.52
Free T$_3$	724	0.00	−0.02, 0.01	225	0.00	−0.01, 0.02	499	−0.01	−0.03, 0.01	0.30

Abbreviations: CI, confidence interval; T$_3$, triiodothyronine; T$_4$, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH, thyroid-stimulating hormone.

[a] Some participants were missing thyroid hormone measurements due to insufficient sample volume.

[b] All models adjusted for age at thyroid hormone measurement and age at thyroid hormone measurement squared.

[c] Coefficients represent the mean (free T$_3$, free T$_4$, and total T$_4$) or percent (TSH) change in thyroid hormone level for each 10-fold increase in serum TCDD concentration.

[d] Percent change in serum TSH concentration was calculated using the following formula: $(10^\beta - 1) \times 100$.

TCDD and 1996 free T$_3$ (β = −0.02, 95% CI: −0.04, 0.00), the association was not statistically significant (*P* = 0.13). Thyroid hormone levels in 1996 or 2008 were not associated with 1996 TEQ (Table 4). A large and imprecise inverse association was found between 1996 TEQ and 2008 TSH (β = 19.2, 95% CI: −16.4, 69.9), which was particularly strong among women who were premenarche at the time of the explosion (β = 28.4, 95% CI: −29.6, 134.2; *P* for interaction = 0.12), but effect modification was not significant.

Estimates of causal effects using marginal structural models led to similar conclusions as those drawn from multiple linear regression (see Web Tables 2 and 3). A 0.43-μg/dL decrease in 1996 total T$_4$ (95% CI: −0.82, −0.05) was observed for every 10-fold increase in 1976 TCDD concentrations; this relationship was stronger among women who were premenarche at the time of the explosion (β = −0.89, 95% CI: −1.54, −0.24; *P* for interaction = 0.02) relative to those who were postmenarche (β = −0.10, 95% CI: −0.32, 0.11).
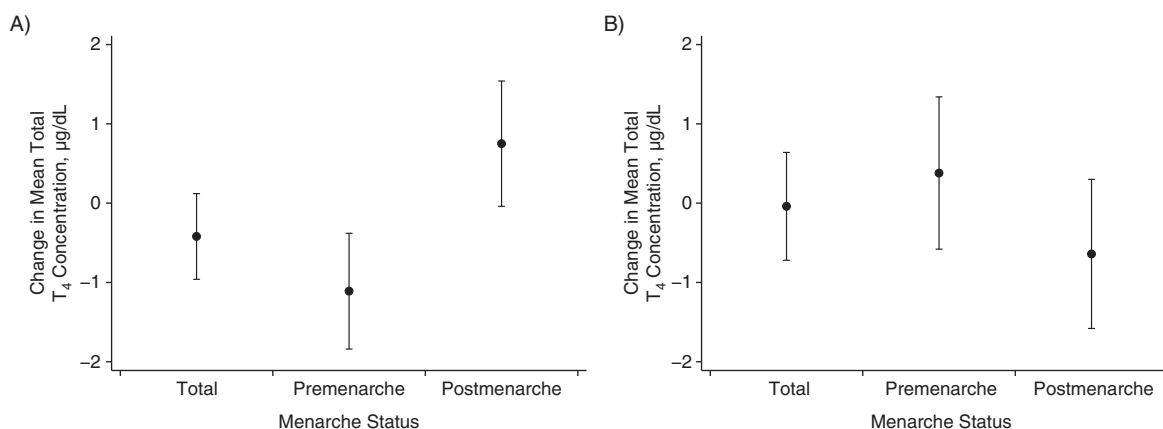

Figure 1. Associations between 1976 (A) and 1996 (B) serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) concentrations and 1996 serum total thyroxine (T$_4$) concentrations by menarcheal status at the time of the explosion (*n* = 251), Seveso Women's Health Study, Italy, 1976–2009. Results were based on multiple linear regression models including exposure at both time points. Coefficients represent the mean change in total T$_4$ concentration for each 10-fold increase in TCDD. Models adjusted for age at thyroid hormone measurement and age at thyroid hormone measurement squared.

Table 3. Associations Between 1996 Log$_{10}$ TCDD Concentration (Parts per Trillion) and Thyroid Hormone Levels by Menarcheal Status at the Time of the Explosion (1976), Seveso Women's Health Study, Italy, 1976–2009

| Thyroid Hormone | No. of Participants[a] | Adjusted[b] β[c] | 95% CI | Menarcheal Status at Explosion | | | | | | P for Interaction |
| | | | | Premenarche | | | Postmenarche | | | |
				No.	Adjusted[b] β[c]	95% CI	No.	Adjusted[b] β[c]	95% CI	
1996 Thyroid Hormone Measurements										
TSH[d]	260	−4.46	−18.89, 12.54	161	−8.94	−25.14, 10.75	99	−1.12	−26.48, 32.94	0.62
Total T$_4$	251	−0.37	−0.90, 0.17	153	−0.54	−1.32, 0.20	98	−0.11	−0.85, 0.64	0.42
Free T$_4$	251	−0.03	−0.16, 0.10	153	0.00	−0.14, 0.13	98	−0.04	−0.31, 0.22	0.74
Free T$_3$	252	−0.02	−0.04, 0.00	153	−0.01	−0.04, 0.01	99	−0.02	−0.05, 0.02	0.85
2008 Thyroid Hormone Measurements										
TSH[d]	235	11.89	−4.28, 30.32	146	14.66	−10.09, 46.21	89	2.71	−17.58, 28.00	0.17
Total T$_4$	236	−0.09	−0.63, 0.45	146	0.16	−0.54, 0.87	90	−0.44	−1.28, 0.39	0.26
Free T$_4$	237	0.01	−0.05, 0.06	146	0.02	−0.05, 0.09	91	−0.01	−0.10, 0.07	0.52
Free T$_3$	237	0.01	−0.01, 0.02	146	0.01	−0.01, 0.03	91	0.00	−0.02, 0.02	0.84

Abbreviations: CI, confidence interval; T$_3$, triiodothyronine; T$_4$, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH, thyroid-stimulating hormone.

[a] Some participants were missing thyroid hormone measurements due to insufficient sample volume.

[b] All models adjusted for age at thyroid hormone measurement and age at thyroid hormone measurement squared.

[c] Coefficients represent the mean (free T$_3$, free T$_4$, and total T$_4$) or percent (TSH) change in thyroid hormone level for each 10-fold increase in serum TCDD concentration.

[d] Percent change in serum TSH concentration was calculated using the following formula: $(10^{\beta} - 1) \times 100$.

Estimated effects of 1996 TCDD on 1996 total T$_4$ (β = −0.76, 95% CI: −1.42, −0.11) and free T$_3$ (β = −0.02, 95% CI: −0.04, −0.01) were significant in marginal models (Web Table 3); inclusion of 1976 TCDD in the model drove estimates towards the null for total T$_4$ (β = −0.45,

95% CI: −1.37, 0.46) but not for free T$_3$ (β = −0.03, 95% CI: −0.06, 0.00). Other estimates were nonsignificant.

Odds ratios for associations between any TCDD/TEQ measures and hyperthyroidism and hypothyroidism in 1996 or 2008 were generally weak, imprecise, and not statistically

Table 4. Associations Between 1996 Log$_{10}$ Total TCDD Toxic Equivalent (Parts per Trillion) and Thyroid Hormone Levels by Menarcheal Status at the Time of the Explosion (1976), Seveso Women's Health Study, Italy, 1976–2009

| Thyroid Hormone | No. of Participants[a] | Adjusted[b] β[c] | 95% CI | Menarcheal Status at Explosion | | | | | | P for Interaction |
| | | | | Premenarche | | | Postmenarche | | | |
				No.	Adjusted[b] β[c]	95% CI	No.	Adjusted[b] β[c]	95% CI	
1996 Thyroid Hormone Measurements										
TSH[d]	260	−7.71	−36.09, 33.27	161	−19.64	−49.56, 28.02	99	−2.35	−47.64, 82.12	0.65
Total T$_4$	251	−0.57	−1.77, 0.64	153	−1.01	−2.81, 0.80	98	−0.23	−1.80, 1.34	0.56
Free T$_4$	251	−0.08	−0.37, 0.22	153	−0.07	−0.39, 0.25	98	−0.05	−0.60, 0.50	0.93
Free T$_3$	252	−0.04	−0.08, 0.01	153	−0.03	−0.09, 0.04	99	−0.04	−0.11, 0.03	0.85
2008 Thyroid Hormone Measurements										
TSH[d]	235	19.17	−16.39, 69.86	146	28.42	−29.58, 134.21	89	−2.53	−339.84, 55.34	0.12
Total T$_4$	236	−0.28	−1.49, 0.92	146	0.24	−1.50, 1.98	90	−0.73	−2.39, 0.94	0.37
Free T$_4$	237	0.06	−0.07, 0.18	146	0.08	−0.10, 0.26	91	0.03	−0.14, 0.21	0.65
Free T$_3$	237	0.02	−0.02, 0.05	146	0.02	−0.03, 0.07	91	0.01	−0.03, 0.06	0.83

Abbreviations: CI, confidence interval; T$_3$, triiodothyronine; T$_4$, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH, thyroid-stimulating hormone.

[a] Some participants were missing thyroid hormone measurements due to insufficient sample volume.

[b] All models adjusted for age at thyroid hormone measurement and age at thyroid hormone measurement squared.

[c] Coefficients represent the mean (free T$_3$, free T$_4$, and total T$_4$) or percent (TSH) change in thyroid hormone level for each 10-fold increase in serum TCDD concentration.

[d] Percent change in serum TSH concentration was calculated using the following formula: $(10^{\beta} - 1) \times 100$.

significant (data not shown). None of the sensitivity analyses substantially altered our results.

DISCUSSION

In this study, we found inverse associations between TCDD concentrations in serum collected shortly after the explosion of a trichlorophenol manufacturing plant and total serum T_4 levels in women approximately 20 years later. This association was limited to women who were premenarcheal at the time of the explosion. Among these women, the cross-sectional association between 1996 total T_4 and concurrently measured TCDD was slightly weaker than the longitudinal association with 1976 TCDD and was not statistically significant, possibly due to reduced sample size. This association was substantially weakened when both 1976 and 1996 TCDD were included in the same model, while the association with 1976 TCDD was strengthened. There was some evidence of an association between 1996 TCDD and free T_3, but the estimate was weak. Marginal structural models yielded similar results, confirming these findings. The difference between the longitudinal and cross-sectional associations did not appear to be caused by selection bias due to loss to follow-up, since adjustment for censoring using inverse probability weighting did not materially affect the results. Taken together, these results suggest that serum TCDD concentrations were inversely associated with total T_4 levels in women exposed before menarche.

As previously discussed, studies investigating associations between lipophilic chemicals and thyroid hormones may be affected by reverse causality (36). TCDD and other dioxin-like compounds are highly nonpolar and thus sequester primarily in lipids (37). Thyroid hormones, on the other hand, influence lipid metabolism and serum lipid concentrations (38). Serum TCDD concentrations may therefore be affected by thyroid hormone levels. Inconsistent results from previous studies of TCDD and other dioxin-like compounds may be due to reverse causality, since they all either were cross-sectional, relied on TCDD concentrations in serum collected several years postexposure, or considered ongoing exposure for which the time ordering of exposure and outcome could not be clearly established. The present study was characterized by unique features in that the timing of exposure was precisely known (allowing us to exclude participants who had thyroid disease before the explosion), serum TCDD concentrations were measured shortly after exposure, and the magnitude of exposure would not be expected to have been related to thyroid status when it occurred, thereby limiting the possibility of reverse causation. In this context, our finding of an inverse longitudinal association between TCDD and total T_4 provides a new form of support for the effects of this chemical on thyroid function.

The fact that the cross-sectional association of 1996 TCDD and total T_4 approached the null when 1976 TCDD was included in the models suggests that the initial exposure, rather than continuously elevated serum TCDD concentrations, was primarily associated with total T_4 levels. This has implications for the understanding of the toxicological mechanism of action through which TCDD may affect total T_4 concentrations. TCDD is generally believed to reduce serum T_4 levels

through the induction of uridine diphosphate glucuronosyltransferase in response to binding to the aryl hydrocarbon receptor, resulting in accelerated T_4 clearance (39). Under this mechanism, continuous exposure would be expected to primarily affect T_4 levels. The fact that we found no cross-sectional association may thus suggest that another mechanism of action is at play, perhaps resulting in long-term injury. This possibility of an alternative mechanism is supported by evidence that TCDD affects T_4 levels in aryl hydrocarbon-receptor-null mice (40). In addition, our finding of an inverse association with total T_4 but not free T_4 suggests that the concentration of transport proteins may be affected by TCDD. Experimental animal studies lend some support to this hypothesis. Decreases in total T_4 levels have been consistently reported in laboratory studies conducted in rodents, although those were also generally accompanied by reduction in free T_4 levels (4–6). In addition, TCDD has been shown to competitively displace T_4 from transthyretin, a T_4 transport protein, possibly resulting in increased elimination (41).

Of particular interest is the fact that we found stronger evidence of associations with 1996 thyroid hormone among women who were premenarche at the time of the Seveso explosion, suggesting a specific window of susceptibility. The hypothalamic-pituitary-thyroid axis is known to be particularly sensitive to endocrine modulation before puberty. For instance, the prepubertal axis is estimated to be 6–15 times more sensitive to estrogen than the adult feedback mechanism (42). It is therefore conceivable that TCDD, which has antiestrogenic properties (43), may have a greater effect on thyroid function before puberty. Similar to our findings, serum TCDD was reported to be associated with altered menstrual cycle characteristics among those who were premenarche, but not among those who were postmenarche, at the time of the Seveso explosion (30). It is not clear, however, why this same pattern was not apparent in 2008.

This study had several strengths, including its unique design, which allowed us to investigate both longitudinal and cross-sectional associations. We also had data that allowed us to exclude study participants who had thyroid disease before the explosion, thereby precluding the possibility of reverse causation at the onset of follow-up. In addition, we were able to conduct a number of sensitivity analyses, including an investigation of possible selection bias due to loss to follow-up using inverse probability weighting, and investigated relationships using both standard conditional linear regression and marginal structural models, confirming the robustness of our results. To our knowledge, this is also the first study to have specifically investigated associations between exposure to TCDD and thyroid hormone levels in women.

Limitations include the lack of data on iodine intake, which is an important determinant of thyroid function, and the lack of data on thyroid autoantibody levels, since autoimmune disease is the primary cause of hypothyroidism. However, in the context of the Seveso accident, iodine intake would not be expected to be associated with exposure to TCDD, and we had data on diagnosed thyroid disease. Finally, since only free (unbound) T_4 is bioavailable to bind to the thyroid receptor, the health consequences of a reduction in total T_4 with no change in free T_4 are unclear.

In summary, we report an inverse association between serum TCDD concentrations measured shortly after the Seveso trichlorophenol plant explosion and total T_4 (but not free T_4, free T_3, or TSH) levels measured approximately 20 (but not 30) years later among women who were premenarche at the time of the accident. However, we found no clear associations between 1996 serum TCDD concentrations and any 1996 or 2008 thyroid hormone measures, suggesting an effect of initial exposure rather than of later body burden.

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