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Relationship of Serum TCDD Concentrations and Age at Exposure of Female Residents of Seveso, Italy

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In 1976, a chemical plant explosion near Seveso, Italy, resulted in the highest known exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in residential populations. In 1996, we initiated the Seveso Women's Health Study (SWHS), a historical cohort study of females who were ≤ 40 years old at the time of explosion and residents of the most heavily contaminated areas, zones A and B. Serum samples collected near the time of the explosion were analyzed for TCDD. We also analyzed pooled serum samples collected in 1976 from females who resided in zone non-ABR, the "unexposed" zone, to assess concurrent background exposures to other dioxins, furans, and coplanar polychlorinated biphenyls (PCBs). The median lipid-adjusted TCDD level for residents of zones A and B combined was 56 ppt (range = 2.5–56,000 ppt). Zone A residents had 5-fold higher TCDD levels (*n* = 67, median = 272 ppt) than did zone B residents (*n* = 814, median = 47 ppt). The youngest children had the highest TCDD levels, which decreased with age at explosion until approximately 13 years of age and were constant thereafter. Therefore, children living in zones A and B received a disproportionately higher exposure to TCDD as a result of the explosion. Zone of residence and age were the strongest predictors of TCDD level. Chloracne, nearby animal mortality, location (outdoors vs. indoors) at the time of explosion, and consumption of homegrown food were also related to serum TCDD levels. The serum pools from zone non-ABR residents had an average TCDD concentration of 20.2 ppt, and average total toxic equivalent (TEQ) concentration of 100.4 ppt. Therefore, background exposure to dioxins, furans, and PCBs unrelated to the explosion may have been substantial. As a consequence, previous SWHS studies that considered only TCDD exposure may have underestimated health effects due to total TEQ concentrations. *Key words:* biomarkers, children, dioxin, exposure, TCDD, tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 112:22–27 (2004). doi:10.1289/ehp.6573 available via *http://dx.doi.org/* [Online 20 October 2003]

About noon on Saturday, 10 July 1976, an explosion at the ICMESA chemical plant near Seveso, Italy, approximately 25 km north of Milan, exposed the nearby residents to the highest known residential exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, or dioxin) (Mocarelli et al. 1988). The explosion, due to an uncontrolled exothermic reaction (Bertazzi and di Domenico 1994), resulted in the release of an aerosol cloud containing sodium hydroxide, ethylene glycol, sodium trichlorophenate, and up to 30 kg of TCDD (Mocarelli et al. 1990). This cloud was deposited over an 18-km^2 area stretching as far as 6 km south-southeast of the plant (di Domenico et al. 1980b).

The contaminated area was divided into three major zones (A, B, and R) in decreasing order of surface soil concentrations of TCDD (Bisanti et al. 1980). Zone A, with 15.5–5,477 μg/m^2 TCDD in surface soil samples, sustained an almost immediate 25% animal mortality rate. Residents of zone A (*n* = 736, representing ~211 families) were evacuated within 20 days of the explosion. Most families (*n* = 152) were able to return to their homes by 1977, after the least contaminated areas of zone A had been decontaminated (Needham et al. 1997/1998). The residents of zone B (*n* = 4,737), with surface soil TCDD levels ranging from < 5 to 43.8 μg/m^2, were not evacuated but were warned about the risk of consuming locally grown produce and meat. Children up to 12 years of age and pregnant women were relocated out of the area on a daily basis, and breast-feeding was strongly discouraged (Mocarelli et al. 1992). Acute TCDD intake among residents of zone B was presumed to occur primarily through dust inhalation and dermal absorption and was estimated to be 10- to 100-fold less than that received by zone A residents (Mocarelli et al. 1991a). Zone R, with surface soil TCDD levels ranging from < 5 to 9.7 μg/m^2, had about 30,000 residents. These residents were neither evacuated nor warned about ways to reduce exposure. Zone non-ABR, an area of more than 180,000 residents surrounding the other zones, was designated as the "unexposed" area. The few soil samples taken near the border between zone non-ABR and zone R were all below the level of detection (di Domenico et al. 1980a).

Significant TCDD exposure to the population was demonstrated by the occurrence of 193 cases of chloracne, a specific but not sensitive indicator of exposure to dioxin-like compounds (Mocarelli et al. 1991a, 1991b). Almost all cases of chloracne (88%) were diagnosed among children younger than 15 years of age, and the 19 most severe cases were among children from zone A. Investigators hypothesized that the higher frequency of chloracne in children was due to the greater opportunity for TCDD exposure from ingestion of contaminated garden produce and other substances, from inhalation of contaminated air through outdoor activities, and from dermal contact with soil, vegetation, and dirt outside the home (Bertazzi and di Domenico 1994; Mocarelli et al. 1991a). Among children living in the same building and even within the same family, some developed chloracne while others did not. Until recently, however, little information has been available on the actual exposures to children (Needham et al. 1997/1998).

A health assessment of the population was initiated soon after the explosion. As part of this effort, blood samples were collected for clinical chemistry tests, and small volumes of serum were stored for later analyses. The existence of archived serum samples has made it possible to quantify individual TCDD levels soon after the explosion (Mocarelli et al. 1988). TCDD analyses of sera for zone A residents (*n* = 178) and for a small sample of zone B residents (*n* = 35) have suggested that children, in particular females, who were younger than 13 years of age in 1976 had higher TCDD levels than did older residents (Needham et al. 1997/1998, 1999). However, these results may not have been representative of the population, because there was preferential analysis of sera

from those with chloracne, from those thought to have the highest exposure based on soil levels, or from those later diagnosed with cancer.

TCDD has been classified as a human carcinogen [International Agency for Research on Cancer (IARC) 1997] and has the potential to disrupt multiple endocrine pathways (Grassman et al. 1998; Safe 1995). Because substantial animal and limited human evidence has suggested that *in utero* and early childhood exposure may affect reproductive health (Birnbaum 1995; Brown et al. 1998; Chaffin et al. 1996; Eskenazi et al. 2002b; Gray and Ostby 1995; Heimler et al. 1998; Mocarelli et al. 2000; Murray et al. 1979; Nau et al. 1986; Petroff et al. 2000; Roman and Peterson 1998), 20 years after the explosion we initiated the Seveso Women's Health Study (SWHS), a historical cohort study to evaluate the association of TCDD exposure and reproductive outcomes among female residents. As part of this investigation, stored serum samples were analyzed for TCDD from a sample of the female population residing in zones A and B. In this report, we further describe the relationship of serum TCDD and age and examine the relation of serum TCDD to other correlates of exposure. We also report background exposures to dioxins, furans, and polychlorinated biphenyls (PCBs) during the same time period as measured in pooled serum samples from female residents of zone non-ABR.

Materials and Methods

Study population. Eligible for enrollment in the SWHS cohort were 1,271 women who were ≤ 40 years of age in 1976, who had adequate stored sera collected between 1976 and 1980, and who resided in the most heavily exposed areas, zones A or B, at the time of the explosion in 1976. The archived sera samples represent approximately 95% of zone A residents and about 60% of zone B residents, with the lowest proportion in zone B from the youngest age group, 0–5 years (~35%). Enrollment for SWHS began in March 1996 and was completed in July 1998. More than 95% of the women were located 20 years after the explosion, and about 80% (*n* = 981) participated. A human subjects protocol was approved by institutional review boards at participating institutions, and informed consent was obtained from all women before participation. Participation rates did not differ by zone. We analyzed the serum only for those who participated in the SWHS.

Procedure. Details of the study procedure are presented elsewhere (Eskenazi et al. 2002b). Questionnaires assessing demographic, lifestyle, reproductive, pregnancy, and medical histories, and exposure information were administered by trained interviewers who were blind to the zone of residence of the women. The women did not know their individual serum TCDD levels at the time of interview, and 20% of women reported that they did not know their zone of residence.

Laboratory analyses. Archived serum samples from residents of zones A and B had been collected from July 1976 through 1985 and stored at –20°C in the Desio Hospital laboratory. For analysis, we preferentially selected the first sample available that was of adequate volume (> 0.5 mL) and that was collected between 1976 and 1981. The samples were sent on dry ice to the U.S. Centers for Disease Control and Prevention, where they were measured for TCDD by high-resolution gas chromatography/mass spectrometry (Patterson et al. 1987). Values were reported on a lipid-weight basis in parts per trillion by dividing TCDD on a whole-weight basis by total serum lipid content, estimated from measurements of triglycerides and total cholesterol (Akins et al. 1989). The median serum sample weight for these samples was 0.65 g, and the median lipid-adjusted limit of detection was 18.8 ppt.

For 899 of the 981 zone A and B women (92%), TCDD was measured in sera collected between 1976 and 1977. For 54 women (5%), TCDD was measured in sera collected between 1978 and 1981. For 28 women (3%), the volume of archived serum specimen was inadequate for analysis; therefore, a serum sample collected in 1996 was analyzed. For women with post-1977 TCDD values that were detectable but ≤ 10 ppt (*n* = 4), the measured value was retained for analysis. For women with post-1977 TCDD levels > 10 ppt, the TCDD exposure level was back-extrapolated to 1976, according to the Filser model (Kreuzer et al. 1997) for women ≤ 16 years of age in 1976 (*n* = 27), and according to the first-order kinetic model for older women (*n* = 42) (Pirkle et al. 1989). For nondetectable values (*n* = 96), a serum TCDD level equal to one-half the detection limit was assigned (Hornung and Reed 1990).

Because serum volumes from zone A and B residents were only adequate to measure TCDD, we pooled archived sera from female residents who resided in zone non-ABR in 1976 to determine the background levels of dioxins, furans, and PCBs during the same period. Based on available archived serum, a total of nine pools were created for three age groups: 0–12 years (two pools), 12–20 years (three pools), and 20–40 years (four pools). Each pool consisted of approximately 1 mL serum from 20 or 21 females for a total of about 20 mL of serum. These pools were analyzed for 22 polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar PCBs (PCB congeners 77, 81, 126, 169) by high-resolution gas chromatography/ mass spectrometry methods (Patterson et al. 1987). In addition, 36 PCBs were measured, including the mono-*ortho*-substituted PCBs (PCBs 105, 118, 156, 157, 167) that have a toxic equivalency factor (TEF) greater than zero (Van den Berg et al. 1998). Values were reported on a lipid-weight basis in parts per trillion (Akins et al. 1989).

Statistical analyses. Statistical analyses were performed using Stata 7.0 (Stata 2001). Because the distribution of serum TCDD level was approximately log-normal, serum TCDD was logarithmically transformed (base 10). We graphically present the relation of log TCDD concentration to age at the time of explosion by means of a robust nonparametric Lowess curve. We categorized age at explosion into five age categories based on developmental stages (0–2, > 2–5, > 5–10, > 10–13, and > 13 years).

We used multiple linear regression to examine the relation of serum log TCDD levels to other exposure-related covariates, including report of chloracne, consuming homegrown produce, finding dead animals on their property, and being indoors versus outdoors at the time of the explosion. We performed separate analyses by zone of residence (A, B). We tested for any differences in the relative importance of these covariates in the two zones by fitting all two-way interactions with zone in a single model. Similarly, we tested for interactions of other covariates with age in a piecewise model with age considered linear up to age 13 and constant thereafter. For all regressions, we report the adjusted ratio of geometric means and nonparametric (Huber, sandwich) standard errors, which are valid even when conventional assumptions for regressions, such as constant residual standard deviation, are violated (Huber 1967).

For the pooled background samples from zone non-ABR, we present the mean and standard deviation across all pools. The total toxic equivalent (TEQ) for the pooled specimens was calculated as the sum of the product of each analyte concentration and its TEF (Van den Berg et al. 1998).

Results

A histogram of the distribution of serum TCDD levels for the group of 981 women from zones A and B is presented in Figure 1A. The median serum TCDD was 55.9 ppt, lipid-adjusted, with a range from 2.5 to 56,000. In Figure 1, B and C present the distribution of serum TCDD by zone of residence. The 167 women from zone A had a median lipid-adjusted serum TCDD level of 272.0 ppt, with a range from 3.2 to 56,000 ppt. The 814 women from zone B had a median serum TCDD level of 47.1 ppt (range = 2.5–3,140), about one-fifth the median of zone A residents (*p* < 0.001).

Figure 2 presents the Lowess plot of serum TCDD levels by age at time of explosion for the 981 women. From the plot it is apparent that the shape of the curve is approximately

piecewise linear. Serum TCDD levels are highest among the youngest females and decrease until approximately 13 years of age, after which there is no change.

Descriptive statistics for selected characteristics for the full cohort and by zone of residence are presented in Table 1. The median serum TCDD levels of females were highest for children ≤ 2 years of age (median = 288 ppt) and decreased to a median of 44 ppt for children > 13 years of age ($p < 0.001$). The highest levels were observed at the youngest ages in both zones A and B. Women who reported having been diagnosed with chloracne had a higher median serum TCDD level (median = 1,575 ppt) than those who reported having another family member with chloracne (median = 257 ppt), who in turn had a higher median value than those who reported no one in their household with chloracne (median = 53 ppt). Women who reported that animals died on their home property had a higher median TCDD serum level (median = 135 ppt) than women who did not report any animal mortality (median = 49 ppt). The relationships of serum TCDD with chloracne and with animal mortality were stronger in zone A than in zone B. Women who reported being outdoors at the moment of the explosion also had a higher median TCDD level (median = 76 ppt) than women who were indoors at the moment of the explosion (median = 63 ppt). Women who reported consuming homegrown foods after the explosion had a higher median serum TCDD level (median = 69 ppt) than those who did not (median = 47 ppt).

In univariate regression analysis, the zone of residence, age at explosion, chloracne diagnosis, nearby animal mortality, location at the time of the explosion, and consumption of homegrown food were all independently significantly positively related with serum TCDD ($p < 0.01$). Age explained 7.3% and zone of residence explained 22.0% of the total variance (R^2) in log TCDD concentration.

The results of multivariate regression models for the full cohort and stratified by zone of residence are presented in Table 2. In multivariate analysis, residence in zone A, younger age, chloracne, nearby animal mortality, outdoors at the time of explosion, and consumption of homegrown food were all positively related to log TCDD levels ($p < 0.001$) after adjustment of the other variables. The multivariate model explained 40.2% of the total variance; therefore, including other variables in the model with zone explained 18.2% more variance than that explained by zone alone. Age and zone were the strongest predictors of serum TCDD level. Age at time of explosion explained 14.5%, and zone explained 13.8% (partial r^2) of the variance unexplained by other factors.

As presented in Table 2, for the full cohort, residents of zone A had a nearly 4-fold higher

adjusted geometric mean TCDD level than zone B residents. Mean TCDD levels decreased with age until 13 years of age; the adjusted ratios of the geometric means decreased from 4.4 for females who were ≤ 2 years of age to 1.2 for females who were between 10 and 13 years of age, relative to those > 13 years of age. Women who reported a diagnosis of chloracne had a more than 3-fold higher adjusted

geometric mean, and women who reported a family member being diagnosed with chloracne had a 2-fold higher adjusted geometric mean than those who reported no chloracne. The adjusted geometric mean ratio was increased for recall of nearby animal mortality after the explosion (ratio = 1.4), location outside at the time of explosion (ratio = 1.2), and consuming homegrown foods (ratio = 1.3).
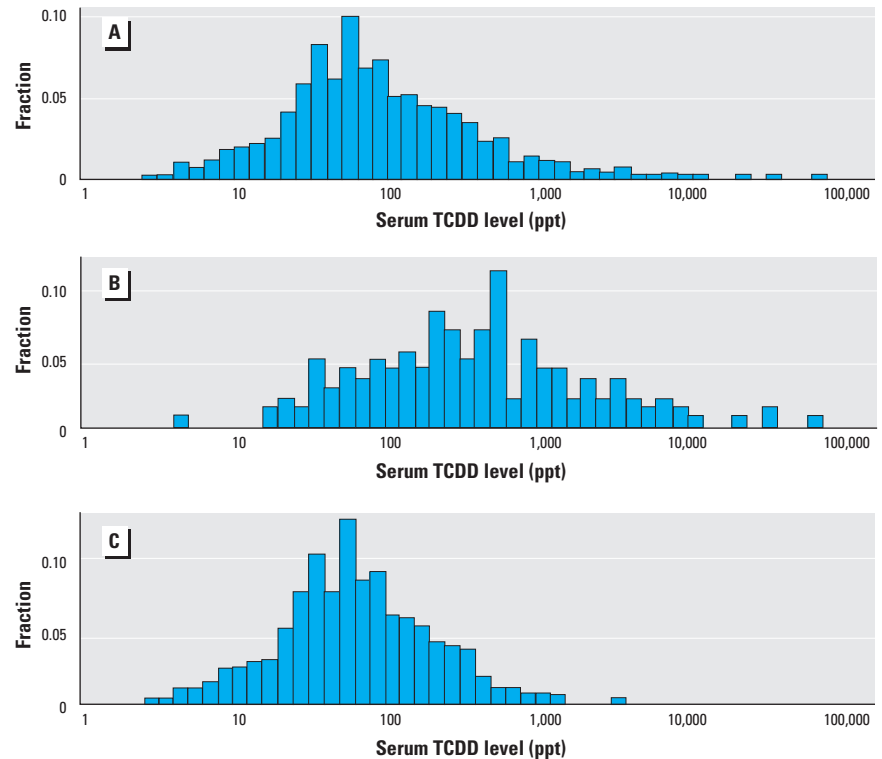
Figure 1. Distribution of 1976 serum TCDD levels for (*A*) the full SWHS cohort (*n* = 981; median = 55.9 ppt; interquartile range = 28–157 ppt; range = 2.5–56,000), and residents of (*B*) zone A (*n* = 167; median = 272 ppt; interquartile range = 92–883 ppt; range = 3.2–56,000), and (*C*) zone B (*n* = 814; median = 47.1 ppt; interquartile range = 25–106 ppt; range = 2.5–3,140).
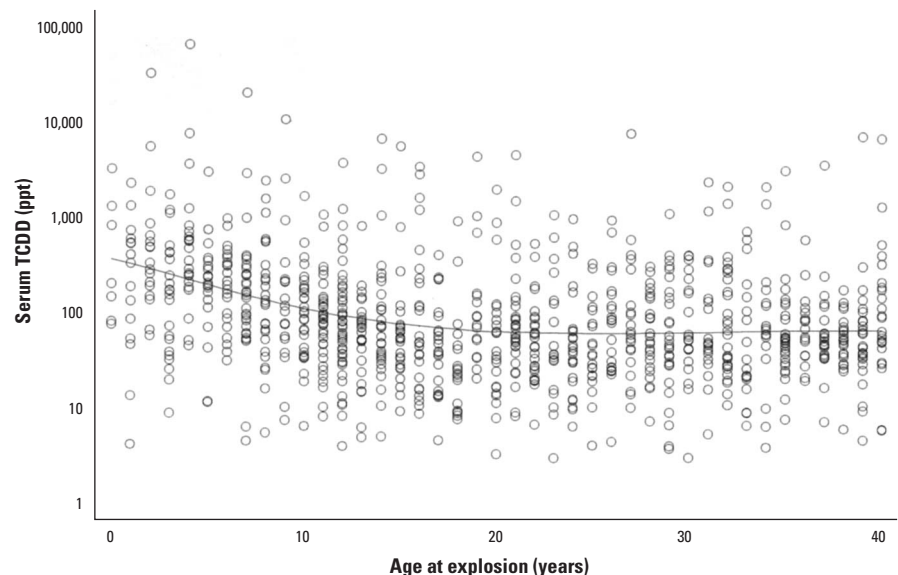
Figure 2. Lowess plot of serum TCDD concentration versus age at explosion for SWHS cohort.

Somewhat different associations appear in the zone-specific analyses in Table 2. The age trend was weaker in zone A than in zone B (p-value for difference in age slopes = 0.06). We found a significant interaction of zone and chloracne (p < 0.02). TCDD levels were related to diagnosis of chloracne only in zone A.

We also examined the interaction of age at explosion with each of the other covariates in the multivariate model (data not shown). We found a significant interaction between age and homegrown food consumption (p = 0.005). Although younger women had higher levels of TCDD regardless of their consumption of homegrown food, the age difference was

smaller among those who consumed homegrown foods (data not shown).

Table 3 presents a summary of TCDD levels and total TEQ levels in serum pools from 1976 residents of zone non-ABR. Overall, the average TCDD level was 20.2 ppt and the average total TEQ was 100.4 ppt. The contribution to total TEQ by analytes other than TCDD

Table 1. Distribution of serum TCDD level by covariates and by zone of residence.

| | Total | | | | Zone A | | | | Zone B | | | |
| | | TCDD | | | | TCDD | | | | TCDD | | |
Covariate	n (%)	GM	Median	IQR	n (%)	GM	Median	IQR	n (%)	GM	Median	IQR
Total population	981 (100)	69	56	28–157	167 (17)	306	272	92–883	814 (83)	51	47	25–106
Age at explosion (years)[a],*												
>0–2	40 (4)	310	288	118–670	15 (9)	780	553	221–2,820	25 (3)	178	180	76–417
>2–5	66 (7)	200	208	98–433	14 (8)	662	546	182–1,500	52 (6)	145	174	69–300
>5–10	126 (13)	118	123	50–251	17 (10)	457	322	192–1,420	109 (13)	96	104	47–196
>10–13	111 (11)	61	64	31–117	19 (11)	177	141	70–492	92 (11)	49	53	26–86
>13	638 (65)	52	44	24–99	102 (61)	249	242	81–770	536 (66)	39	39	22–70
Chloracne diagnosed[a],*												
Yes, participant	30 (3)	762	1,575	144–3,180	20 (12)	2,253	2,700	1,575–6,150	10 (1)	87	137	30–247
Yes, family member	47 (5)	241	257	42–1,050	26 (16)	704	778	333–1,752	21 (3)	64	43	28–140
No	890 (91)	60	53	27–131	119 (72)	189	199	77–460	771 (96)	50	47	25–102
Animal mortality[a],*												
Any	179 (18)	166	135	46–443	76 (46)	534	476	175–1,741	103 (13)	70	61	31–143
None	771 (79)	57	49	25–121	86 (52)	207	208	65–484	685 (84)	48	45	24–99
Don't know	30 (3)	73	56	41–119	5 (3)	57	38	23–41	25 (3)	77	71	45–119
Location at accident[a],*												
Outdoors	192 (20)	97	76	38–219	35 (21)	586	770	119–1,960	157 (19)	65	61	34–135
Indoors	600 (61)	74	63	29–169	108 (65)	306	284	124–687	492 (60)	54	50	27–115
Don't know	42 (4)	40	46	26–98	3 (2)	119	230	31–6,590	39 (5)	34	45	22–98
Consume homegrown food[a],*												
Yes	418 (43)	82	69	36–171	88 (53)	311	302	122–826	330 (41)	58	54	31–111
No	543 (55)	60	47	24–140	75 (45)	296	237	63–864	468 (57)	47	42	22–100
Don't know	18 (2)	96	83	45–348	4 (2)	422	766	321–952	14 (2)	66	67	45–100

IQR, interquartile range.
[a]Numbers may not total 981 because of missing data. *p < 0.05 (ANOVA significant difference in $\log_{10}$TCDD by covariate).

Table 2. Adjusted[a] ratio of geometric means: multiple regression models of $\log_{10}$TCDD levels.

| | Full SWHS cohort (n = 981) | | | Zone A (n = 167) | | Zone B (n = 814) | | Interaction with zone |
Covariate	Partial r^2 (%)[b]	Ratio[c]	95% CI	Ratio	95% CI	Ratio	95% CI	p-value
Zone of residence	13.8							
A		3.7	2.9–4.6					
B[d]		1.0						
Age at explosion (years)	14.5							
>0–2		4.4	2.7–7.0	2.5	1.0–6.1	5.1	2.9–8.7	0.17*
>2–5		3.5	2.5–4.8	1.5	0.6–4.2	4.1	3.1–5.6	0.06**
>5–10		2.5	2.0–3.0	1.8	0.9–3.4	2.7	2.1–3.3	
>10–13		1.2	1.0–1.5	0.8	0.5–1.5	1.3	1.0–1.6	
>13[d]		1.0		1.0		1.0		
Chloracne diagnosed	4.1							
Yes, participant		3.3	1.7–6.4	8.0	3.5–18.3	0.9	0.5–1.8	<0.02
Yes, family member		2.0	1.3–3.2	3.2	1.6–6.4	1.3	0.8–2.1	
No[d]		1.0		1.0		1.0		
Animal mortality	1.3							
Any		1.4	1.2–1.8	1.6	1.0–2.7	1.3	1.1–1.6	0.15
None[d]		1.0		1.0		1.0		
Location at accident	4.2							
Outside		1.2	1.0–1.4	1.2	0.6–2.3	1.1	0.9–1.3	0.54
Inside[d]		1.0		1.0		1.0		
Consume homegrown food	1.4							
Yes		1.3	1.1–1.5	1.1	0.7–1.6	1.4	1.2–1.6	0.83
No[d]		1.0		1.0		1.0		
Total R^2		41.0%		40.0%		23.8%		

Abbreviations: CI, confidence interval; df, degrees of freedom; SS, sum of squares.
[a]Covariates in the model include zone of residence, age at explosion, chloracne diagnosis, animal mortality, location at accident, and consumption of homegrown food. [b]Additional variation contributed by the individual factor with the other factors in the model; partial r = 1 – (residual SS full)/(residual SS reduced). [c]Adjusted for all other variables. [d]Reference group.
*p-Value for hypothesis of equal adjusted geometric mean ratios in Zone A and Zone B (df = 4). **p-Value for hypothesis of equal slopes up to 13 years of age in Zone A and Zone B (df = 1).

averaged 80.2 ppt. The TCDD levels were highest for the youngest age groups, averaging around 40 ppt, whereas the oldest groups had the highest contribution to the total TEQ from analytes other than TCDD.

Discussion

The SWHS is the first study to examine exposure at the time of the explosion in females residing in zones A and B near Seveso who were not selected because of disease status or other exposure-related factors. We found a 5-fold difference in serum TCDD levels between females residing in zone A (median = 272 ppt) and those residing in zone B (median = 47 ppt). Children were exposed disproportionately, with the youngest children receiving the greatest exposure. We also found higher levels of TCDD in serum pools from the youngest residents of zone non-ABR. Although the explosion at the ICMESA plant may have resulted in exposure specifically to TCDD, the results of samples from residents of zone non-ABR also suggest that the background exposure to other dioxins, furans, and PCBs may have been substantial. However, the limited data available suggest that these background exposures were within the range of levels for other areas of Europe around the same period (Päpke et al. 1994).

Previous studies of exposure conducted in Seveso reported slightly higher TCDD levels for zone A female residents (median = 409 ppt) (Needham et al. 1997/1998, 1999) but included women ≥ 40 years of age at the time of the explosion and women who did not participate in the SWHS. They also reported higher TCDD levels for the youngest residents of zones A and B, but this was based on sera from a select sample of 35 zone B residents (Needham et al. 1997/1998, 1999). Landi et al. (1998) estimated that the serum TCDD levels at the time of explosion averaged about 230 ppt for zone A and 48 ppt for zone B residents based on back-extrapolation of TCDD measured in 58 serum samples collected in 1996. These extrapolations are remarkably close to the actual values we measured in immediate postexplosion sera.

The higher levels of TCDD seen in younger children are consistent with the higher lead (Brody et al. 1994; Pirkle et al. 1998) and pesticide levels (Adgate et al. 2001) observed in children living in the same environment as adults. Children, proportionate to their weight and surface area, consume more water, food, and air than adults (National Research Council Committee on Pesticides in the Diets of Infants and Children 1993). This implies that children will have substantially heavier exposures to any toxicants present in their environment. Children also differ substantially from adults in the sources, pathways, and routes of exposure (Needham and Sexton 2000), and these exposure parameters are likely to change over the course of childhood. For example, crawling infants may have increased dermal contact with contaminated floors; toddlers often put their hands and objects in their mouths; and school age children spend a significant proportion of their time at school or playing outdoors (Sexton et al. 2000). Thus, any chemical that settles closer to the ground, such as TCDD in Seveso, could potentially result in higher exposure in children, especially among younger children with more hand-to-mouth contact.

Higher serum levels in the younger children may have also been due to exposure from breast-feeding. However, women in zones A and B were strongly advised against breast-feeding, and only three women report having been breast-fed as a child in the period after the explosion. Excluding these three women from the final model does not change the relationship of age and TCDD levels. Given that only about 60% of females from zone B provided blood, our results may be biased, especially if only the young children whose parents suspected higher levels of exposure gave blood; however, the age differential was also present in zone A, where almost 100% of residents provided samples. It is difficult to assess the causes for the age-related differences because reports of exposure factors 20 years after the event (e.g., recall of homegrown food) are subject to error, especially in the youngest children, and were often based on the adults' retelling of the events around the explosion. Although a biomarker of exposure such as serum TCDD levels allows for

the integration of dose over all exposure routes and pathways (Needham and Sexton 2000), we can only speculate as to how the exposure occurred and why it resulted in changing levels during childhood and compared with adults.

The higher body burdens of children compared with adults may, in part, explain their higher frequency of chloracne. Indeed, we find that the 30 children with chloracne had higher TCDD levels than did other children living within the same zone. Children may also be more sensitive to the effects of the TCDD exposure. For example, soon after the explosion, slight alterations in serum γ-glutamyl-transferase and alanine aminotransferase activity were observed in children 6–10 years of age (younger children were not studied) (Mocarelli et al. 1986) but not in adults (Mocarelli et al. 1991a). Also, we observed perturbations in menstrual cycle length only in those exposed before puberty (Eskenazi et al. 2002b).

It is particularly curious that the levels of TCDD were highest in the pooled samples from children of zone non-ABR (0–12 years of age), although they were lower than those for children from zones A and B. The levels for children in zone non-ABR (33.4 and 47.6 ppt) were similar to the median (45 ppt) previously reported in pooled serum samples from children < 13 years of age from zone R (Needham et al. 1997/1998). One possible reason for the relatively high levels in the "unexposed" zone is nonrandom selection of blood samples in that zone. The Hospital of Desio offered to analyze the clinical chemistries for all those residing in the outlying areas. Bias may have resulted if parents who were most concerned about exposure requested a blood draw for their child. For example, a parent who did not reside in the exposed zones may have been more likely to agree to a blood draw if their child had been nearby at the time of the explosion or if the child had consumed food products from the area.

Previous studies that have assessed exposure to TCDD in human populations have been hampered by the lack of biologic measures of exposure (Constable and Hatch 1985; Le and Johansson 2001; Rylander et al. 1995, 2000) or used serum samples collected years after the exposure (Michalek et al. 1998a, 1998b). The serum samples analyzed from the Seveso cohort are unique because most were collected soon after the explosion. Most of these archived sera (> 800 specimens) were not analyzed until the initiation of the SWHS. Thus, this report provides some of the first evidence, in a sample of the Seveso population, of the high levels of TCDD exposure incurred by the population residing in the most heavily exposed zones. We also present the TCDD levels (20 ppt) for those residing during the same period in the "unexposed" areas, which

Table 3. Summary of TCDD and TEQ levels in pooled serum from 1976 residents of zone non-ABR.

Age group (years)	TCDD (ppt)	Contribution to TEQ by other PCDDs, PCDFs, PCBs (ppt)[a]	Total TEQ (ppt)
0–12	47.6	71.9	119.5
0–12	33.4	80.2	113.6
12–20	17.1	58.7	75.8
12–20	22.2	52.8	75.0
12–20	20.0	79.4	99.4
20–40	10.1	91.6	101.7
20–40	8.7	117.4	126.1
20–40	11.6	80.7	92.3
20–40	11.3	88.7	100.0
Mean ± SD	20.2 ± 12.9	80.2 ± 18.9	100.4 ± 17.7

[a]Data from Van den Berg et al. (1998).

were similar to those previously reported (~15 ppt) (Needham et al. 1997/1998). Many previous studies of the Seveso cohort have used zone to estimate exposure (Bertazzi et al. 2001; Fara and Del Corno 1985; Mastroiacovo et al. 1988; Mocarelli et al. 1986). Given that zone explained only 22% of the variance of serum TCDD levels, exposure based on zone alone may result in misclassification. Using other exposure-related variables such as age could substantially improve classification in cases where serum levels have not been measured.

This study provides for the first time evidence of background exposure (in zone non-ABR) to relatively high levels of other dioxins, furans, and PCBs that contribute to total TEQ. The pooled data indicate that analytes other than TCDD contribute 80 ppt, on average, to the total TEQ. If these background levels are similar in all zones, individuals with low levels of TCDD might still have substantial total TEQ levels. Our previous studies of health outcomes considered only TCDD exposure (Eskenazi et al. 2002a, 2002b; Mocarelli 2001; Warner et al. 2002). Because we considered only TCDD, our results may have underestimated the health effects due to total TEQ exposure.

In summary, female residents of the area near Seveso, Italy, at the time of the 1976 explosion were exposed to high levels of TCDD. Serum TCDD levels were related to a number of factors, particularly zone of residence and age. It is likely that the overall total TEQ levels of these residents resulted not only from their TCDD levels but also from substantial background exposure to dioxins, furans, and PCBs that were probably unrelated to the explosion.

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