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The Seveso accident: A look at 40 years of health research and beyond

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Abstract

A 1976 chemical factory explosion near Seveso, Italy exposed residents to high levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin). Dioxin is a known human carcinogen and potent endocrine disruptor. It is highly lipophilic and has a long half-life in humans. Much of what we know and can learn about the risks of dioxin exposure on human health arose from the tragic circumstances of Seveso. This review aims to describe the Seveso accident, summarize the results of 40 years of research on the health of the Seveso population since the accident, and discuss next-stage research on the health of Seveso residents, their children, and grandchildren.

Keywords

Dioxin; TCDD; Seveso; epidemiology; disaster

Introduction

In July 1976, a chemical plant explosion near Seveso, Italy exposed locals to the highest known levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) exposure to a residential population (Mocarelli 2001; Pesatori and Bertazzi 2012). Dioxin has been classified as a known human carcinogen by the International Agency for Research on Cancer (IARC 1997). It is a potent endocrine disruptor, highly lipophilic, and extremely stable with a long half-life in humans of 7 to 9 years (IARC 1997; Needham et al. 1999; Needham et al. 1997). Because it is a combustion byproduct, it is a widespread environmental contaminant

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in industrialized areas and background exposures, while declining, are ubiquitous. Much of what we know and can learn about the risks of dioxin exposure on human health arose from the tragic circumstances of Seveso. The purpose of this paper is to describe the Seveso accident, to summarize the results of 40 years of research on the health of the Seveso population after the accident, and to discuss next-stage research on possible health effects among the residents, their children, and grandchildren.

The History of the Seveso Accident in 1976

On Saturday, 10 July 1976 at 12:37 PM, a chemical reactor exploded at the ICMESA plant located in Meda near Seveso, Italy (25 km north of Milan) (see previous detailed descriptions (Mocarelli 2001; Pocchiariet al. 1979)). The plant was manufacturing 2,4,5-trichlorophenol, an intermediary for cosmetics and pharmaceuticals. A runaway chemical reaction resulted in the release of an aerosol cloud that included sodium hydroxide, ethylene glycol, sodium trichlorophenate, and an estimated 15 to 30 kg of TCDD over an 18-km² area (di Domenico et al. 1980). Area residents in the path of the aerosol cloud developed nausea, headaches, eye irritation, and 19 children were admitted to the local hospitals with skin lesions (Signoriniet al. 2000). In the ensuing weeks, the area experienced high animal and plant mortality, and nearly 200 cases of chloracne were reported among residents, mostly among children (Assennato et al. 1989).

On 25 July, a multipronged study was launched under the sponsorship of the Regione Lombardia Italy. The bioclinical investigations team from the Hospital of Desio collected blood specimens from thousands of residents for immediate clinical chemistry tests. The researchers stored remaining serum for future analyses. Individuals who provided blood samples were followed up regularly until 1982 and periodically thereafter. Health effects assessed included neurological problems, infection, dermatologic lesions, and pregnancy and child health (Mocarelliet al. 1986).

In the initial absence of individual biomarker-based exposure levels, health studies from Seveso relied upon zones of residence to classify levels of exposure. The zones of residence matrix was constructed based on systematic sampling and measurement of surface soil TCDD levels. The region with the highest surface soil TCDD levels (range: 15.5 – 5,477 µg/m²) was classified as zone A (Mocarelliet al. 1991b). The nearby area was divided into zone B, zone R, and zone non-ABR (soil TCDD not detected; considered to be unexposed) (see Figure 1) (Mocarelliet al. 1991a). Zone A housed 736 residents (212 families), all of whom were evacuated beginning two weeks (from July 26th to August 2nd) after the explosion; these residents underwent immediate medical examination and clinical laboratory tests. Those that lived in the most heavily exposed areas within zone A were not allowed to return to their homes, which were considered contaminated and later destroyed as part of the clean-up (Ghezzi et al. 1982). Zone B, the area of next greatest contamination, housed almost 5,000 residents who were not evacuated but were warned against consuming locally-grown produce and poultry; these residents also received a medical examination and clinical laboratory tests. In addition, pregnant women and children less than 12 years from zone B were relocated out of the area on a daily basis. Zone R, the least contaminated area, housed about 32,000 residents who were warned not to consume local foods (Mocarelliet al. 1992).

Although soil dioxin levels have been used as a proxy of exposure, these levels do not reflect variation in individual-level frequency and duration of contact with dioxin-contaminated media. Seveso residents are the only dioxin-exposed population for whom blood samples were collected near the time of exposure, enabling a more accurate exposure assessment of absorbed dose. In 1987, eleven years after the Seveso accident, the Centers for Disease Control and Prevention (CDC) developed an analytical method (using high-resolution gas chromatography/high-resolution mass spectrometry) to measure TCDD concentration in human serum (Patterson et al. 1987). This new method and the nearly 30,000 stored samples at the University Laboratory of the Hospital of Desio opened the door for more precise measurements of individual exposure to be examined in relation to the health of the population.

Seveso Women's Health Study (SWHS)

In 1996, 20 years after the explosion, a historical cohort study was initiated of the female residents (newborn to 40 years old in 1976) of zones A or B who had blood collected near the time of the explosion. The Seveso Women's Health Study (SWHS), funded by the United States National Institutes of Health (NIH) and Regione Lombardia Italy, is the only comprehensive study to date of the health of a female population exposed to TCDD. It is unique in being a large cohort with a wide range of TCDD exposure documented by individual-level serum TCDD measurements. The methods for the SWHS have been summarized previously (Eskenazi et al. 2000)

A total of 981 women participated in the first follow-up of the population (1996). The majority of women were from zone B (83%). At the time of the explosion, women in SWHS averaged 20.1 (± 11.3) years of age, 71% were postmenarcheal, 38% were parous, and <1% were postmenopausal (see Table 1). The women were followed up again in 2008 (Warner et al. 2011) and 2014; participants did not differ from the full SWHS cohort with respect to characteristics at explosion (see Table 1). In 2014, the Seveso Second Generation Study was launched, with the goal of characterizing health impacts of *in utero* dioxin exposure in the children of SWHS participants. A total of 943 liveborn children (453 females, 490 males) who were born to 574 SWHS mothers after the explosion were enumerated and ranged in age from newborn to 39 years. A total of 611 children (66.4% of 920 alive and eligible) born to 402 mothers completed the study visit. The 611 child participants averaged 23.7 (range: 2–39) years of age and 51% were female.

Details of data collected for the SWHS and the Second Generation cohorts at each followup study are summarized in Figure 2. For example, for the SWHS women the most recent data collection at the 2014 visit included a fasting blood draw for clinical chemistries and future studies, anthropometric and blood pressure measurements, a personal interview that included questions about her reproductive and medical history, and collection of medical records. For women with children less than 18 years, the interview also included questions about the health of their children as well as demographic and lifestyle factors and medical history. For children 2 to 6 years, participation included a fasting blood draw for clinical chemistries and future studies, and anthropometric measurements. Participation for children 7 to 17 years old included a fasting blood draw, anthropometric and blood pressure measurements, a battery

of computerized neuropsychological tests (Rey's Auditory Verbal Learning, Raven's Progressive Matrices, Connor's Continuous Performance, Wisconsin Card Sort) (Muriel Lezak 2012), and an online self-administered questionnaire including smoking, alcohol, and caffeine habits (10 to 17 years only). Participation for children 18 years or older included fasting blood draw, anthropometric and blood pressure measurements, a personal interview that included the EPIC food frequency questionnaire (Pisaniet al. 1997), and collection of medical records.

Below, we review results from multiple studies on the exposure characteristics and health of the Seveso population, including for residents who resided in the Seveso area at the time of the explosion (denoted as F_0) and the next generation of children born post-explosion (denoted as F_1).

Dioxin Exposure in Seveso Residents (F_0)

In 1988, the CDC measured TCDD in 30 archived serum samples collected in 1976 from 10 zone A residents who developed chloracne and 10 residents who did not develop chloracne, and 10 residents of zone non-ABR (CDC 1988; Mocarelliet al. 1991b). Results of these initial serum measurements confirmed overt exposure to TCDD in zone A residents. The TCDD levels were of similar magnitude to those reported in 2,4,5-trichlorophenol production workers and some were among the highest ever reported (Patterson et al. 1989). Although some of the highest serum TCDD levels occurred in residents who developed chloracne, no TCDD threshold level for chloracne was apparent. Individual susceptibility in addition to dose likely played a role in who developed chloracne.

CDC performed additional TCDD measurements in archived serum from male and female residents of zone A (n=296), zone B (n=80), zone R (n=48), and zone non-ABR (11 pools, 4–10 individuals/pool) (Needham et al. 1999; Needham et al. 1997). Although no details on sample selection were provided, results indicated residents of zone A had higher median TCDD levels than zone B, who had higher levels than zone R. Within zones, serum TCDD levels were higher among those who were younger (<13 years of age) at time of explosion but did not differ by sex. The non-ABR pools (median = 15 ppt) were similar to background dioxin levels in 1976. Finally, serial TCDD measurements in serum collected between 1976 and 1982 for a sample of zone B residents suggested there was no additional TCDD exposure as a consequence of living in zone B after the explosion. The higher serum dioxin levels in Seveso could have resulted from inhalation of airborne fallout, dermal contact, ingestion of contaminated foods, or possibly other dioxin exposure (Needham et al. 1999).

Of the 981 women in the SWHS cohort, 167 were from zone A and 814 were from zone B. Although blood had been collected and archived from area residents soon after the explosion, the CDC had previously measured TCDD in only a few hundred samples. As part of the SWHS, hundreds more were measured, providing important insight into the exposure of the Seveso population. Details of the serum sample selection and TCDD concentrations measured in 1976 serum have been presented elsewhere (Eskenazi et al. 2004a; Eskenazi et al. 2000).

The median lipid-adjusted serum TCDD concentration in blood samples from 1976 for the SWHS cohort was 55 parts per trillion (ppt) and ranged from 2.5 to 56,000 ppt (Eskenaziet al. 2004b). Median serum TCDD levels were significantly higher among residents of zone A than zone B (272 ppt vs. 47 ppt), but there was wide variation in TCDD exposure within zones (zone A, 3.2 to 56,000 ppt; zone B, 2.5 to 3,140 ppt) and considerable overlap in TCDD levels between zones. Thus, these results suggested that using zone of residence as the primary measure of TCDD exposure information could readily lead to exposure misclassification. Age at the time of the explosion was the only other factor related to initial serum TCDD levels. Those less than 10 years of age had higher serum levels.

In addition to determining the background levels of TCDD (as previously presented in (Needhamet al. 1999; Needhamet al. 1997), another objective was to determine the background exposure levels of other dioxins, furans, and dioxin-like polychlorinated biphenyls (PCB) unrelated to the accident. Serum samples collected in 1976 from female residents of the non-ABR zone were pooled (9 pools, 20–21 females/pool). The TCDD level in serum pools averaged 20.2 ppt, and the levels of other dioxin-like compounds (dioxins, furans, polychlorinated biphenyls) averaged 80.2 ppt, resulting in an average total dioxin toxic equivalency (TEQ) (Van den Berget al. 1998), of 100.4 ppt (Eskenaziet al. 2004b). (N.B.: It was not possible to measure other dioxinlike compounds in the very small volumes of archived 1976 serum samples in the SWHS cohort.) Thus, although the Seveso explosion resulted in exposure specifically to TCDD, the results of pooled serum measurements revealed background-level exposure to other dioxin-like chemicals in this population, similar to background levels reported in other European countries during this period (Papkeet al. 1994).

Individual TCDD and total TEQ (Van den Berget al. 2006), were measured in archived 1996–98 serum (referred to as “1996” samples) for the subset of SWHS women (n=266) who reported a live birth between 1994 and 2009 (Warneret al. 2014). This subset was younger at exposure (mean = 10.2 ± 5.8 years) and had higher 1976 TCDD levels than the full SWHS cohort, though levels ranged widely. For this subset, 1996 serum TCDD and TEQ levels remained elevated (median = 7.3 and 25.7 ppt, respectively) relative to those measured in serum collected from unexposed female residents in 1998–2000 (median = 1.6 and 18.1 ppt, respectively) (Warneret al. 2005). The elevated total TEQ found among SWHS participants was due primarily to elevated TCDD. In the SWHS cohort, TEQ concentrations for other congeners were not elevated above background-exposed female residents in 1996. Thus, 20 years after the initial exposure, TCDD concentrations in this SWHS sample, the majority of whom were children in 1976, remained relatively elevated. Initial 1976 serum TCDD levels and age at explosion were the strongest predictors of 1996 serum TCDD. The apparent TCDD elimination half-life was 7.1 years for those >10 years old in 1976, but shorter in younger age groups (<5 years old = 4.3 years; 6–10 years old = 5.2 years). The difference in elimination by age may result from dilution due to child growth and increased body mass, as well as hepatic elimination at the higher exposure doses (Warneret al. 2014).

There is some evidence the half-life of TCDD may be longer in women than men. Needham et al. reported a longer TCDD half-life in women (~9 years) than men (~7 years) based on serum TCDD concentrations measured in serial samples collected between 1976 and 1993

for 27 Seveso residents (12M, 15F) (Needhamet al. 1999; Needhamet al. 1997). Also, TCDD levels were measured in serum collected almost twenty years after the accident for 62 Seveso zone B residents (Landiet al. 1998); geometric mean serum TCDD levels were significantly higher in women (17.6 ppt) than men (6.7 ppt), even after adjustment for additional covariates. Taken together with findings from the SWHS, there is evidence that the half-life of TCDD in Seveso may be age- and sex-related.

Based on clinical and animal studies suggesting that the elimination rate of TCDD is dosedependent (Michaleket al. 2002), Emond et al. developed a physiologically based pharmacokinetic (PBPK) model in rodents, which uses body-burden-dependent elimination rates and includes physiologic compartments for liver, adipose tissue, rest-of-the-body (maternal), and two pregnancy-related compartments, the placenta (maternal/fetal) and fetus (Emondet al. 2004). The model was optimized for humans using data from U.S Air Force veterans who participated in Operation Ranch Hand, and a single volunteer who ingested a dose of 1.14 ng TCDD/kg and was followed for 40 days (Emondet al. 2005). Emond et al. modified the PBPK model to include pregnancy and lactation using repeated TCDD serum measurements from the SWHS data. The modified PBPK model accurately predicted blood TCDD levels during the 20 years post-exposure, including for periods of pregnancy and lactation; in fact, findings from the model's validation suggested that gestation and lactation do not significantly impact the maternal elimination rate (Emondet al. 2016).

Health Effects of Dioxin Exposure in Seveso Residents (F₀)

Health of F₀ as Children

Several studies have focused on the health of the exposed children (see Table 2 for summary).

Chloracne.—While the exact number of identified chloracne cases has varied across reports (Assennatoet al. 1989; Caramaschiet al. 1981; Pocchiariet al. 1979), by most estimates nearly 200 cases were diagnosed among area residents, with the majority in children less than 15 years of age (Assennatoet al. 1989; Baccarelliet al. 2005; Caramaschiet al. 1981). The highest rate was found in children who resided in zone A, where 48.1% of children were diagnosed with chloracne. It is not clear whether the children were more susceptible to the chloracnegenic effects of TCDD or whether they had greater TCDD exposure (Sweeney and Mocarelli 2000). TCDD levels remained higher in 101 chloracne cases compared to 211 controls at the time of follow-up between 1993 and 1998, and chloracne risk was highest in those who were younger than 8 years at the time of the explosion and had TCDD concentrations at follow-up >10 ppt (OR = 7.4; 95% CI: 1.8, 30.3) (Baccarelliet al. 2005).

Liver function—In an early study of Seveso children with and without chloracne, 2.8% of children with chloracne had out-of-range serum γ -glutamyltransferase levels (a measure of detoxification activity), compared to none without chloracne (Caramaschiet al. 1981). Between July 1976 and June 1982, Mocarelli et al. performed 20 different clinical chemistry tests, including liver function and lipid metabolism in serial blood samples from 1,500 children who were 6 to 10 years of age at the time of the explosion and who resided in zones

A, B or R (Mocarelliet al. 1986). Small but significant differences were found between zones A and B and zone R for serum γ -glutamyltransferase and alanine aminotransferase levels in boys between 1976 and 1977, but not in the following years. In girls, a difference in serum aspartate aminotransferase levels was found between 1980 and 1982. In 31 zone A children, 6 to 8 years old, children with chloracne had significantly elevated levels of urinary d-glucaric acid, a sensitive index for hepatic microsomal drug/xenobiotic-metabolizing enzyme induction, in samples collected between August and September 1976 (Ideoet al. 1982). By 1981, urinary d-glucaric acid levels for all children were in the normal range (Ideoet al. 1985).

Immune function.—Between 1976 and 1979, 48 zone A children and unexposed controls underwent repeated immunologic tests (Mocarelliet al. 1991a; Sirchia, 1982). Children from zone A exhibited higher values for lymphocyte responses to phytohemagglutinin (PHA) and to pokeweed mitogen (PWM), perhaps indicating dysregulated cellular immunity.

Tooth development.—In a sample of 48 children with a median serum TCDD of 476 ppt who were less than 9.5 years of age in 1976, high serum TCDD (>700 ppt) was related to developmental aberrations in the teeth, including hypoplastic enamel and missing lateral incisors (Alaluusuaet al. 2004).

Reproductive Health Outcomes in F₀

Male fertility.—Twenty-two years after the explosion, Mocarelli et al. examined semen quality and reproductive hormone levels in 135 men who were 1 to 26 years of age in 1976 and lived in exposed zones at the time of the explosion as well as 184 unexposed controls (22–47 years at semen collection) (Mocarelliet al. 2008). Men who were youngest at the time of exposure (1 to 9 years old, median 1976 serum TCDD = 210 ppt) had significantly lower sperm concentration (mean = $53.6 \times 10^6/\text{mL}$ vs. $72.5 \times 10^6/\text{mL}$; $p = 0.03$), percent progressive motility (mean = 33.2% vs. 40.8%; $p < 0.001$), and total motile sperm count (mean = 44.2×10^6 vs. 77.5×10^6 ; $p = 0.02$) than similar-aged unexposed men. In contrast, men who were older at the time of exposure (10 to 17 years, median 1976 serum TCDD = 164 ppt) had significantly higher total sperm count and total motile sperm count than the similar-age unexposed group; no significant differences were found in men who were over 17 years in 1976. In addition, men exposed at ages 1–17 years, but not in older age groups, had significantly lower serum estradiol levels and higher follicle stimulating hormone levels than unexposed men in similar age groups. These data suggest that men exposed to TCDD prior to puberty may have adverse effects on their semen quality while those exposed around or just after puberty may have better semen parameters.

Female fertility.—In the SWHS, Eskenazi et al. examined the relationship between TCDD exposure and time to pregnancy for the women's first post-explosion conception resulting in a live birth (Eskenaziet al. 2010). TCDD exposure was examined as measured serum concentration circa 1976 and as estimated concentration at the time of "trying to become pregnant" extrapolated from 1976 concentrations. Fertility among women was reduced with increasing TCDD serum concentrations. A 10-fold increase in serum TCDD concentration in 1976 was associated with a 25% increase in time to pregnancy (adjusted fecundability OR

= 0.75; 95% CI: 0.60, 0.95) and almost double the odds of infertility (> 12 months to become pregnant) (adjusted OR = 1.9; 95% CI: 1.1, 3.2). Similar results were found when TCDD serum concentrations were extrapolated to the time of pregnancy.

Menstrual cycle characteristics, age at menarche, and ovarian function.—In a follow-up case-control study of chloracne cases 20 years after the explosion, no significant difference in irregular menstrual cycle was noted, although the sample size was small (Baccarelliet al. 2005). Menstrual cycle characteristics were examined among the larger sample of pre-menopausal SWHS women (Eskenaziet al. 2002b). Stratification by menarcheal status in 1976 revealed that women who were premenarcheal at the time of the explosion experienced an almost 1 day increase in cycle length per 10-fold increase in 1976 TCDD concentrations (adjusted β = 0.93; 95% CI: -0.01, 1.86), while no such association was seen for women who were postmenarcheal when exposed (adjusted β = -0.03; 95% CI: -0.61, 0.54) (p -interaction = 0.08). Women who were premenarcheal at the time of the explosion also had reduced odds of scanty flow (adjusted OR = 0.33; 95% CI: 0.10, 1.06), whereas women who were postmenarcheal did not (adjusted OR = 1.36; 95% CI: 0.70, 2.64) (p -interaction = 0.03). There was no association with number of days of flow. In addition, TCDD serum levels were related to early menarche in those who were youngest at explosion, albeit nonsignificantly (Warner and Eskenazi 2005; Warner et al. 2004).

Although TCDD was associated with an elongation of the menstrual cycle in SWHS, there was no evidence that TCDD exposure was associated with ovarian dysfunction (Warner et al. 2007). TCDD was not associated with the number of follicles on ultrasound, odds of having any follicles > 10 mm, size of the dominant follicle, odds of ovulation, or estrogen or progesterone levels by TCDD (in women who had ovulated).

Endometriosis—In the SWHS, 601 women who were less than 30 years old at the time of explosion underwent a pelvic exam, transvaginal ultrasound and, if medically indicated, a laparoscopy. In addition, detailed information about pelvic pain, dysmenorrhea, and dyspareunia was collected. A total of 19 women (3.2%) were classified as cases (14 with surgically-confirmed diagnosis, and 5 diagnosed by ultrasound) (Eskenaziet al. 2002a). Compared to the lowest dose group (< 20 ppt), the relative risk ratio (RRR) of the moderate dose group (20.1–100 ppt) was 1.2 (90% CI: 0.3, 4.5) and the RRR of the highest dose group was 2.1 (90% CI: 0.5, 8.0) (p -trend = 0.25).

Fibromas.—At the 1996 SWHS follow-up, 251 (26.3%) had fibromas based on study ultrasound, medical record, or self-report (Eskenaziet al. 2007). The age-adjusted hazard ratio associated with a 10-fold increase in serum TCDD was 0.83 (95% CI: 0.65, 1.07), suggestive of an inverse association between the hazard of fibroid onset and increasing exposure. Age-adjusted hazard ratios for women with medium (20.1–75.0 ppt) and high (20.1–75.0 ppt) TCDD levels were 0.58 (95% CI: 0.41, 0.81) and 0.62 (95% CI: 0.44, 0.89), respectively, suggesting a lower hazard of having fibromas.

Age at onset of menopause.—In the SWHS, Eskenazi et al. examined the association between serum TCDD levels and age at menopause at the time of the 1996 follow-up. Of 616 eligible women who were 35 years or older at interview, 169 (27.4%) and 83 (13.5%)

were in natural and surgical menopause, respectively (Eskenazi et al. 2005). In Cox proportional hazards modeling, risk of early menopause trended upward in the first four quintiles of TCDD, but not in the highest quintile. Compared with women in the lowest quintile (Q1), increased risk of early menopause was found for women in Q2 (adjusted hazard ratio (HR) = 1.1; 95% CI: 0.7, 1.8), Q3 (adjusted HR = 1.4; 95% CI: 0.9, 2.3), and Q4 (adjusted HR = 1.6; 95% CI: 0.9, 2.6), but not for women in Q5 (adjusted HR = 1.1 (95% CI: 0.6, 1.9)). These results suggest a non-monotonic dose-related association between serum TCDD levels and earlier onset of natural menopause, up to a serum concentration of about 100 ppt TCDD, but not above.

Chronic Health Outcomes in F₀

Cancer incidence and mortality.—Bertazzi and colleagues have published several papers examining cancer incidence and mortality in relation to zone of residence at the time of the accident (Bertazzi et al. 1993; Bertazzi et al. 2001; Bertazzi et al. 1997; Bertazzi et al. 1992; Bertazzi et al. 1989; Consonni et al. 2008; Pesatori et al. 2009; Pesatori et al. 1993; Pesatori et al. 1998). In the first 25 years following the explosion (1976–2001), overall cancer mortality was not elevated in any exposure zone. However, when the analysis was limited to deaths occurring 20 or more years after the explosion, cancer mortality was increased in zone A (n=18 deaths, rate ratio (RR) = 1.6; 95% CI: 1.0, 2.6) but not in zones B or R (Consonni et al. 2008). Increased mortality from lymphatic-hematopoietic cancers was noted in zones A (n=6 deaths, RR = 2.2; 95% CI: 1.0, 5.0) and B (n=28 deaths, RR = 1.6; 95% CI: 1.1, 2.3), with higher increases reported among females. In a recent update including deaths from 1976–2013, the authors reported that the observed increased mortality from lymphatic-hematopoietic cancers in zones A and B persisted, especially among women (Consonni et al. 2016; Consonni et al. 2008).

Though overall cancer incidence (as distinct from mortality) was not elevated in the first 20 years following the explosion (1976–1996), these researchers observed a non-significant increase in incidence of cancer at a wide range of cancer sites, including breast cancer (n=8 cases, RR = 1.4; 95% CI: 0.7, 2.9), among residents of zone A (Pesatori et al. 2009). Consistent with the mortality studies, incidence of lymphatic-hematopoietic cancers was significantly increased in zone B (n=29 cases, RR = 1.6; 95% CI: 1.1, 2.3) and were also increased, albeit non-significantly, in zone A (n=4 cases, RR = 1.4; 95% CI: 0.5, 3.7).

In the SWHS, Warner et al. examined serum TCDD concentrations measured in the serum collected circa 1976 with overall cancer incidence and breast cancer incidence at 20 years post-explosion (Warner et al. 2002) and 32 years post-explosion (Warner et al. 2011). A significant increased risk for breast cancer incidence (HR = 2.1; 95% CI: 1.0, 4.8) per 10-fold increase in serum TCDD level was found at 20 years post-explosion and a significant, dose-related increased risk for all cancer incidence (HR = 1.8; 95% CI: 1.3, 2.5) was found at 32 years post-explosion. This relationship will be re-examined using the 2014–2016 data, when the majority of the cohort will have reached menopause and breast cancer incidence will likely have peaked.

Cardiovascular disease.—After 25 years (1976–2001), Consonni et al. (2008) reported increased mortality from ischemic heart disease (IHD) among male residents of zone A (n=7 deaths, RR = 2.5; 95% CI: 1.2, 5.2) and increased mortality from hypertension among female residents of zone A (n=4 deaths, RR = 2.6; 95% CI: 1.0, 6.9). Among residents of zone B, mortality from cerebrovascular disease was non-significantly increased in both females (n=50 deaths, RR = 1.2; 95% CI: 0.9, 1.6) and males (n=51 deaths, RR = 1.2; 95% CI: 0.9, 1.6). Among SWHS women, in 2008, a 10-fold increase in 1976 serum TCDD was non-significantly associated with increases in total cardiovascular disease (HR = 1.7; 95% CI: 0.9, 3.2) and IHD specifically (HR = 1.7; 95% CI: 0.8, 3.9) (Warner et al. 2014).

Diabetes, body mass index (BMI), and metabolic syndrome.—Using mortality data from the first 25 years of follow-up (1976–2001), Consonni and colleagues reported excess mortality from diabetes among female residents of all exposure zones, with more marked increases noted in zone B (n=20 deaths, RR = 1.78; 95% CI: 1.14, 2.77) than in zone A or zone R (Consonni et al. 2008). A recent update including deaths from 1976–2013 reported an increase in diabetes-related deaths, mainly among women, with an exposure zone-related gradient [zone A (n=5, RR = 2.1; 95% CI: 0.9, 5.0); zone B (n=24, RR = 1.7; 95% CI: 1.1, 2.5); zone R (n=161, RR = 1.4; 95% CI: 1.2, 1.6)] (Consonni et al. 2016).

In the SWHS, by the 2008 follow-up, 54 women (5.5%) were categorized as diabetic. Warner et al. observed a somewhat decreasing incidence of diabetes with increasing TCDD levels (adjusted HR = 0.8; 95% CI: 0.4, 1.3) (Warner et al. 2013). In line with these findings, a 10-fold increase in serum TCDD was associated with lower body mass index (adjusted β = -0.31; 95% CI: -0.88, 0.27) and a reduced odds ratio for obesity (adjusted OR = 0.8; 95% CI: 0.6, 1.1). However, an increase in metabolic syndrome was observed among women who were exposed early in life (newborn to 12 years of age) (adjusted OR = 2.0; 95% CI: 1.2, 3.3) but not older than age 12 (adjusted OR = 1.0; 95% CI: 0.7, 1.4). A similar difference was found for some but not all individual criteria for metabolic syndrome.

Bone health.—In 2008, 340 women from the SWHS who were <20 years old in 1976 underwent a dual-energy X-ray absorptiometry (DXA) bone scan (Eskenazi et al. 2014). Bone mineral density was measured at the lumbar spine and hip, and hip structural geometry was extracted from hip DXA scans using the Hip Structural Analysis method (Beck 2002). Eskenazi et al. observed no significant associations of TCDD levels with any measure of bone mass density (spine, total hip, femoral neck). However, among premenopausal women, serum TCDD levels were associated with better bone structure in those who were exposed before peak bone mass (considered at 2 years after menarche) (with somewhat stronger associations in those who were exposed before 5 years of age), and in postmenopausal women exposed after peak bone mass (sample sizes for this group however were small).

Thyroid health.—Levels of free and total thyroxine (T4), free triiodothyronine, and thyroidstimulating hormone (TSH) were measured in 909 SWHS women in 1996 and 724 SWHS women in 2008 (Chevrier et al. 2014). Chevrier et al. reported an inverse association of serum TCDD levels circa 1976 with total T4 levels in 1996, with strongest associations among women who were premenarcheal at exposure. By 2008, the association between TCDD levels circa 1976 and total T4, though still inverse, had weakened, and effect

modification by menarche status at explosion was no longer evident. TCDD levels circa 1976 were not associated with levels of any other thyroid hormones, and 1996 serum TCDD levels were not associated with either 1996 or 2008 thyroid hormone levels.

Immune function.—A small study examined molecular markers of immune function and dioxin response using blood samples obtained in 1992 to 1994 from 62 randomly selected subjects from zones A and B and 58 from zone non-ABR (Baccarelli et al. 2004). Plasma TCDD measured nearly 20 years after the accident was negatively associated with plasma IgG levels, a marker of immune defense against microbial challenges, but not with other markers, including IgM, IgA, and complement component C3 and C4 levels (Baccarelli et al. 2002).

Neurologic function.—Initial neurologic screening of zone A residents in 1977 indicated signs of subclinical neurologic damage such as reduced nerve conduction velocity (Pocchiarriet al. 1979). In addition, about 4% of 200 ICMESA plant workers were found to have damage to nerve fibers of multiple (unspecified) nerves (Pocchiarriet al. 1979). Follow-up screening of the chloracne cases in 1985 found no increase in the prevalence of abnormal electrophysiological measures or conduction velocities (Assennatoet al. 1989).

In SWHS, in 1996, physical functioning was assessed in the oldest women (52 to 63 years of age, n=154). No associations were found between serum TCDD exposure and most measures of mobility and dexterity (walking speed, reach down test, coin flipping test); grip strength was the exception with an inverted U-shaped relationship with TCDD but this was examined in a smaller sample (n=98). In 2008, working memory was assessed by the Wechsler digit and spatial span tests in a randomly-selected subset of SWHS women (n=459). No associations were found between serum TCDD and any measure of working memory (Ameset al. 2017a).

Summary of adverse effects of TCDD exposure on F₀.—Table 2 presents a summary of health studies in Seveso residents. Clearly, TCDD exposure resulted in chloracne, and children were most susceptible. The results of Seveso studies also suggest that early-life exposure to relatively high doses of TCDD may have effects on both male and female fertility. However, some of the most compelling evidence is that TCDD exposure increases risk for cancer, and within women, potentially breast cancer. There may be other individual cancers that are increased such as thyroid and blood cancers, but these results are limited by sample size or exposure assessment. TCDD seems to also affect cardiometabolic outcomes including metabolic syndrome, but results on diabetes are conflicting. Evidence for other endpoints examined are less compelling but follow-up may still be warranted of those who were youngest at the time of the accident as they are just beginning to enter middle age.

Dioxin Exposure in Children of Seveso Residents (F₁)

As in research on the health of F₀, TCDD exposure in F₁ generation research also has been determined based on zones of residence and on TCDD concentrations in maternal samples collected circa 1976. In addition, for the Seveso Second Generation cohort, maternal TCDD

and maternal total TEQ has been estimated at the time of pregnancy, which in many cases occurred years or decades post-explosion. Maternal TCDD at pregnancy was estimated by extrapolation from the nearest serum TCDD measure preceding the pregnancy (1976, 1996, 2008) using a first-order kinetic model with a half-life that varied with initial dose, age, and other covariates, as described previously (Warner et al. 2014). For all mothers, the median time between the closest TCDD measure and pregnancy is 4.8 years (IQR: 2.2, 7.4 years), and 87% have a TCDD measurement within one TCDD half-life (9.0 years). Although 1976 specimen volumes were inadequate to assess total TEQ, 1996 and 2008 specimen volumes were adequate for total TEQ analysis; thus, we also examined total TEQ at pregnancy for the subset of births (n=275 livebirths, 343 total births) with 1996 or 2008 measurements. Analyses using maternal TCDD concentrations circa 1976 examine the hypothesis that the primary dose produces a permanent perturbation to the endocrine system or oocytes of exposed women, perhaps via epigenetic mechanisms, which could result in heritable or transmissible changes to offspring (Eskenazi et al. 2003). Analyses using either estimated TCDD or TEQ at time of pregnancy examine the hypothesis that the body burden during the pregnancy is the relevant dose.

Among the 943 liveborn children born to SWHS women between 1976 and 2016, the maternal TCDD level circa 1976 was high [median (IQR) = 60.2 (27.2, 153.0) ppt]. With birth years spanning from 1976 to 2016, estimated maternal TCDD at pregnancy was lower [median (IQR) = 11.9 (5.5, 30.5) ppt], but with a wide range (0.2, 5,730 ppt). For the subset of 275 live births with TEQ measurements, the median estimated maternal TEQ at pregnancy was 22.0 (IQR = 15.6, 29.6; range = 3.7, 634.1) ppt. All three of the second-generation dioxin exposure measures were significantly higher among children of mothers from zone A compared to children of mothers from zone B, but there was wide range of exposure within zones.

Health Effects of *In Utero* Dioxin Exposure (F₁)

In the aftermath of the explosion, there was general concern among the population about the health of unborn children. Because of fear of teratogenicity of TCDD exposure, health authorities advised women to avoid conception (Mastroiacovo et al. 1988). Abortion was illegal at the time of the explosion and some unknown number of women chose to leave the country to abort (Mastroiacovo et al. 1988). Italy legalized abortion in May 1978 and women were able to receive one for psychological and/or social reasons (Tenchini et al. 1983). Because of the unknown number of elected abortions, the risk for spontaneous abortion (SAB) and birth defects from TCDD exposure are potentially underestimated, since women living in highly exposed areas would presumably have been more likely to seek abortion.

Spontaneous abortion.—Early Seveso data, published prior to availability of individual TCDD blood levels, suggested a possible link between zone of residence and SAB (Fara and Del Corno 1985). Rates of SAB were elevated in zones A and B relative to zones R and non-ABR from the last quarter of 1976 through 1977, although variation in rates was hard to interpret due to the small number of pregnancies and that similar variations observed in years before 1977. A cytogenetic study of aborted fetuses from 19 women from the Seveso area and 16 women from outside the area, found a significant increase in the frequencies of

aberrant cells and the average number of aberrations per damaged cell of exposed fetal tissues (Tenchini et al., 1983). Another study of placental morphology noted an unusual ultrastructural appearance in seven of 22 cases, but little information was provided about case selection (Remotti et al., 1981).

In the SWHS, however, maternal TCDD has not been associated with SAB (Eskenaziet al. 2003; Wesselink et al. 2014). Through the most recent follow-up (2014–2016), SWHS women reported 174 post-explosion SABs between July 10, 1976 and 2016 (see Figure 3 for postexplosion birth outcomes summary). As presented in Table 3, a 10-fold increase in maternal initial serum TCDD was not associated with SAB (adjusted Relative Risk = 0.85; 95% CI: 0.69, 1.05). Results were similar for maternal TCDD estimated at pregnancy (adjusted Relative Risk = 0.90; 95% CI: 0.72, 1.12). Results were also similar when the analysis was limited to first post-explosion pregnancies only.

Sex ratio.—Mocarelli et al. examined the sex ratio of children born to zone A residents after the explosion (Mocarelli et al. 1996). Between April 1977 and December 1984, within about one TCDD half-life (~9 years), an excess of female births was reported (26 males versus 48 females, $p < 0.001$). In the subset of 13 couples in which both parents resided in zone A, the nine couples with the highest serum TCDD levels all had daughters (sex ratio: observed 0.76 versus expected 0.514; $p < 0.001$). There was no longer a significant difference in sex ratio for births in the ensuing decade (1985 to 1994). In a larger investigation, based on 674 children born between April 1977 and December 1996, no overall difference in observed versus expected sex ratio (0.487 versus 0.514) was found. However, a decrease in sex ratio (higher proportion of female births) was reported with increasing paternal serum TCDD measures, though only for fathers who were younger than 19 years at the time of explosion (sex ratio = 0.38) (Mocarelli et al. 2000). In contrast, an investigation of chloracne cases ($n=101$) and controls ($n=211$) reported more sons than daughters in chloracne cases for the period up to 20 years after the accident, although the sex ratio was not significantly different for cases and controls (Baccarelli et al. 2005).

Birth defects.—Early Seveso data suggested no association of zone of residence with birth defects (Fara and Del Corno 1985; Mastroiacovo et al. 1988). A congenital malformation registry was established in 1977. Between 1977 and 1983, no increased risk for all birth defects was observed in any of the exposed zones, although the number of births was small, limiting study power, and no infant was diagnosed with a major malformation in zone A (Mastroiacovo et al. 1988). SWHS research has likewise not produced evidence of any positive association of TCDD exposure and birth defects. As of the most recent follow-up (2014–2016), 52 children were born with any birth defect and 13 children were born with a major birth defect (hypospadias/epispadias ($n=2$), anencephaly ($n=1$), anomalies of the heart ($n=5$), anomalies of the vascular system ($n=3$), cleft palate/lip ($n=2$)). While the small number of cases limits statistical power and there is a lack of medical record confirmation, in unadjusted analyses, a 10-fold increase in maternal initial serum TCDD was not associated with having a child with a birth defect (Relative Risk = 1.18; 95% CI: 0.72, 1.91). Results were similar for maternal TCDD estimated at pregnancy (Relative Risk = 1.30; 95% CI: 0.84, 2.02).

Fetal Growth and Length of Gestation.—Among the 943 liveborn children born to 574 SWHS mothers between 1976 and 2016, the average birthweight was 3,241 (± 548) grams, with 7.4% of children born at low birthweight ($< 2,500$ grams). Mean gestational age was 39.1 (± 1.9) weeks with 7.5% of children born preterm (< 37 weeks gestation). Table 4 presents descriptive characteristics, pregnancy-related factors and initial maternal serum TCDD levels for the 574 SWHS women and their 943 post-explosion livebirths.

Table 5 presents the relationship between TCDD exposure and length of gestation and fetal growth. Consistent with previous findings (Eskenazi et al. 2003; Wesselink et al. 2014), a 10-fold increase in maternal serum TCDD circa 1976 was not associated with gestational duration (adjusted $\beta = -0.37$; 95% CI: -1.84, 1.10) or preterm delivery (adjusted OR = 1.18; 95% CI: 0.71, 1.94), or with birthweight (adjusted β per 10-fold increase in TCDD = -38.2; 95% CI: -100.2, 23.7), small-for-gestational-age (adjusted OR = 1.33; 95% CI: 0.92, 1.94), or low birthweight (adjusted OR = 1.40; 95% CI: 0.88, 2.21). When estimated TCDD or TEQ at pregnancy was considered, results were similarly null. For fetal growth measures, limiting the analysis to term births also did not change the results (adjusted $\beta = -37.8$; 95% CI: -96.2, 21.0 for maternal TCDD circa 1976 and adjusted $\beta = -5.1$; 95% CI: -72.4, 62.1 for estimated TCDD at pregnancy). However, there was stronger evidence albeit non-significant of a negative association between maternal TCDD circa 1976 and infant birthweight when analyses were limited to first post-explosion pregnancy (adjusted $\beta = -60.5$; 95% CI: -130.4, 9.5).

Given these borderline significant results that have been consistent at each point of followup, investigations are beginning to focus on whether there might be a particularly susceptible subpopulation within the larger cohort. Current research efforts include examining genetic differences in SNPs along the AhR pathway in the Seveso cohort to determine whether TCDD in a genetically-susceptible subpopulation could result in lowered birthweight (Ames et al. 2017b).

Neonatal thyroid hormone.—Since 1994, TSH has been routinely measured in newborn infant blood as part of the Italian Newborn Screening Program. In the Seveso population, Baccarelli et al. reported that children born between 1994 and 2005 to women from zones A ($n = 56$, geometric mean b-TSH = 1.66 $\mu\text{U/mL}$) and B ($n = 425$, geometric mean b-TSH = 1.35 $\mu\text{U/mL}$) had significantly higher geometric mean b-TSH levels at birth than the children of those living in zone non-ABR ($n=533$, geometric mean b-TSH = 0.98 $\mu\text{U/mL}$) (p -trend < 0.001) (Baccarelli et al. 2008). Similarly, the proportion of neonates with b-TSH levels $> 5 \mu\text{U/mL}$ was higher among zone A (16.1%) and B (4.9%) mothers than non-ABR (2.8%) mothers. In addition, in a small study of 51 mother-child pairs with individual TCDD and TEQ levels measured in blood collected in 1994, a positive association between maternal plasma TCDD and TEQ extrapolated to time of pregnancy and neonatal TSH ($\beta = 0.45$, $p < 0.01$) was observed. However, when women with serum TCDD greater than 50 ppt were excluded, the association between maternal TCDD levels and b-TSH was no longer significant (Baccarelli et al. 2008). Through data collected in the Second Generation Study, reevaluation of this association of maternal serum TCDD with neonatal TSH in a larger sample (over 250 births since 1994) is currently underway.

Male fertility—Mocarelli et al. (Mocarelli et al. 2011) measured sperm quality and hormone concentrations in 39 *in utero* exposed sons born between 1977 and 1984 to mothers who resided in Seveso at the time of the accident and 58 unexposed age-matched male blood donors whose mothers had not lived in the exposed area. *In utero* exposed and breast-fed sons (n=21) had lower sperm concentration ($36.3 \times 10^6/\text{mL}$ vs. $86.3 \times 10^6/\text{mL}$, $p = 0.002$), progressive motility (35.8% vs. 4.2%, $p = 0.03$), and total motile count (38.7×10^6 vs. 98×10^6 , $p = 0.01$) than the unexposed comparison group (n=39). The *in utero* exposed group also had higher follicle stimulating hormone and lower inhibin B than the comparison group. The results support an effect of *in utero* exposure to dioxin on Sertoli Cell proliferation that could lead to permanently reduced sperm quality.

Looking to the Future

Some of the research on the health of the first generation of residents in Seveso (F_0) suggests that those who were youngest at exposure may be more susceptible to the effects of TCDD. Evidence from animal studies concur that early exposure may pose greater effects, which may persist into adulthood. An association with adult-onset diseases, including polycystic ovarian disease, prostate disease, kidney disease (Manikkamet al. 2012b; Nilsson et al. 2012) and mammary cancer (Brown et al. 1998; Fenton et al. 2002) has been reported in rodents exposed to TCDD *in utero*. In addition, TCDD exposure has been shown in animal models to have multigenerational effects. Reduced sperm quality, fertility and increased incidence of premature births have been reported in three generations of offspring of mice exposed to a single dose of TCDD during pregnancy (Bruner-Tran et al. 2014; Bruner-Tran and Osteen 2011; Sanabria et al. 2016). Other multigenerational effects include endometriosis, earlier onset of puberty, increased ovarian cysts, polycystic ovarian disease, and decreased pool size of ovarian primordial follicles in females; increased spermatogenic cell apoptosis and prostate disease in males; and kidney disease (Bruner-Tran et al. 2016; Manikkamet al. 2012a; Manikkamet al. 2012b; Nilsson et al. 2012). In some cases endpoints were noted in the unexposed third generation but not in the exposed first (Manikkamet al. 2012b). Such animal evidence of multi- and trans-generational effects are likely due to epigenetic changes (Bruner-Tran and Osteen 2011; Manikkamet al. 2012b) and demand examination in humans.

As we look forward, we will first examine many of the same outcomes in the children (F_1) as we did in their SWHS mothers to determine whether *in utero* exposure may have resulted in the same or even more profound effects. Specifically, we will examine in the second generation whether *in utero* TCDD exposure is associated with obesity and metabolic syndrome, thyroid function, age at menarche and menstrual cycle characteristics, neuropsychologic function, and fertility. The children are still young, and thus few will have developed chronic diseases such as cancer, diabetes or cardiovascular disease. However, we feel this initial study lays the foundation for ongoing investigation of the long-term health of the Seveso second generation and generations beyond, including elucidation of genetic, epigenetic, and non-genetic mechanisms underlying associations between TCDD exposure and health. Although our research is focused on the Seveso women and their children, the Seveso males and their children should also be followed.

The SWHS provides the only opportunity to look at multigenerational effects of TCDD with biomarkers of exposure. Based on the results of animal studies, we will continue to follow not only the children but the grandchildren of the TCDD-exposed women. We have initiated a preliminary examination of the Seveso third generation (F₂, *i.e.*, the SWHS grandchildren). Of the 431 SWHS children (F₁) who were 18 years or older, 76 daughters have reported 134 pregnancies and 45 sons have reported 76 pregnancies at interview (2014–2016) (see Table 6 for the outcomes of these pregnancies thus far).

Early molecular studies suggest that highly exposed Seveso residents may have altered AhR activity decades after the accident and that genetic variation in P450 enzymes could contribute to susceptibility to TCDD exposure (Landiet al. 2005; Landiet al. 2003). These variants and others in the AHR pathway have been linked to susceptibility to xenobiotics such as PAHs in other populations as well, though few have examined dioxin-like compounds (Brokken et al. 2014; Hung et al. 2013; Sasaki et al. 2006). Because AhR pathway genetics may shape susceptibility, we have begun to examine whether dioxin sensitivity differs by genetic subgroups in SWHS women and their children. As mentioned above, a gene-dioxin study of birthweight is underway with future analyses of additional health endpoints in both the SWHS and their children to follow. Such research may further our understanding of the biological underpinnings not only of TCDD but of other endocrine-disrupting compounds that activate the AhR pathway.

Lessons Learned

An environmental disaster, such as the one in Seveso, is clearly devastating to the local population and introduces potential long-term ecologic, economic, and health consequences both physical and psychological. Unfortunately, a number of environmental disasters, both man-made and natural, have followed the one in Seveso and others will undoubtedly occur in the future. The accident in Seveso provides an important example of the steps epidemiologists and related health professionals can take in the aftermath of a disaster to document its health effects. Epidemiologic research only became possible in Seveso because of the rapid establishment of a long-term health surveillance program of the population, which included - critically - collection and storage of biosamples from a large number of affected individuals despite the fact that methods to analyze exposure in those samples had not yet been developed. Other important steps included careful follow-up of individuals with acute responses to the exposure, in this case, children with chloracne, and development or augmentation of health registries, such as birth defect and cancer registries, to capture information on health effects that could present themselves years after initial exposure. The great degree to which our understanding of the human health effects of dioxin exposure has evolved since 1976 is directly attributable to the foresight of the health professionals who took these critical first steps in Seveso and to the dedicated participation of Seveso families in research studies over the last four decades.

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Abbreviations:

BMI	body mass index
CDC	Centers for Disease Control and Prevention
DXA	dual-energy X-ray absorptiometry
EPIC	European Prospective Investigation into Cancer and Nutrition
IARC	International Agency for Research on Cancer
IHD	ischemic heart disease
NIH	National Institutes of Health
PBPK	physiologically-based pharmacokinetic
PHA	phytohemagglutinin (PHA)
PWM	pokeweed mitogen
SWHS	Seveso Women's Health Study
SNP	single nucleotide polymorphism
SAB	spontaneous abortion
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TSH	thyroid-stimulating hormone
TT4	total thyroxine
TEQ	toxic equivalent

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Highlights

- A 1976 explosion in Seveso, Italy exposed residents to high levels of dioxin.
- Many chloracne cases were diagnosed in Seveso residents, especially in children.
- TCDD levels were associated with decreased male and female fertility in Seveso.
- Increased risk for cancers and cardiometabolic outcomes has been noted.

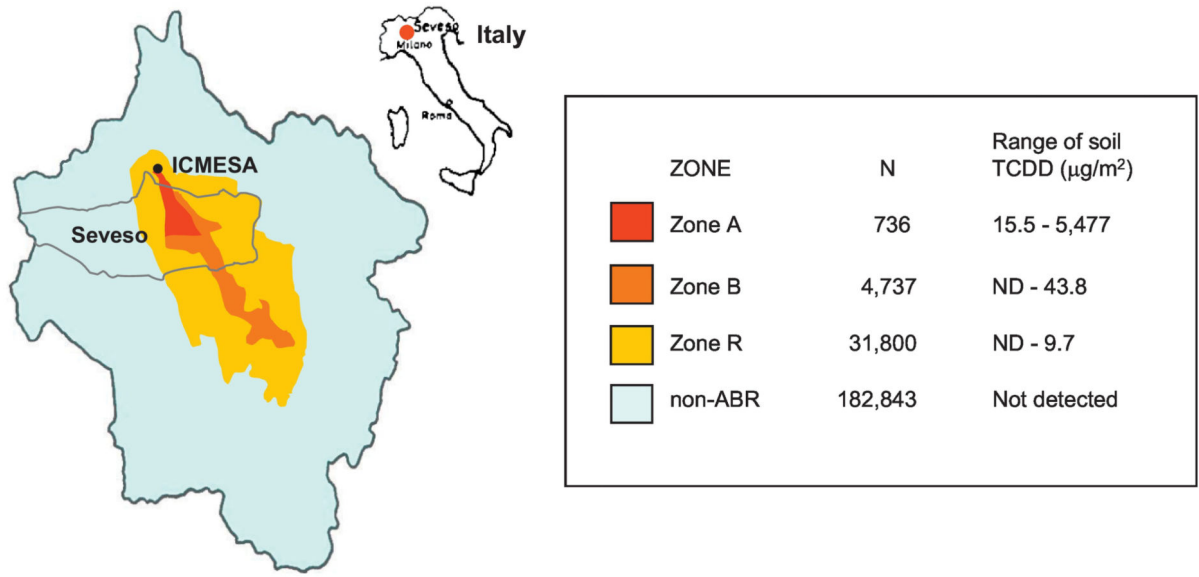


Figure 1. Map of Seveso, Italy indicating the location of Zones A, B, R, and Non-ABR, adapted from (Mocarelliet al. 1991a).

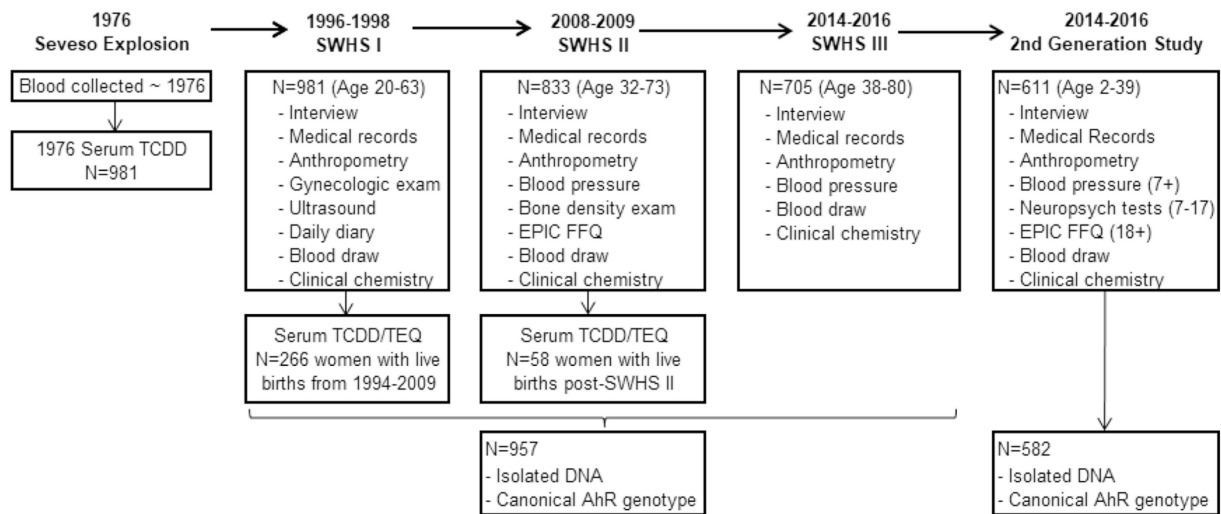


Figure 2. Summary of data collection at each phase of the Seveso Women’s Health and Second Generation Studies, Seveso, Italy 1976–2016

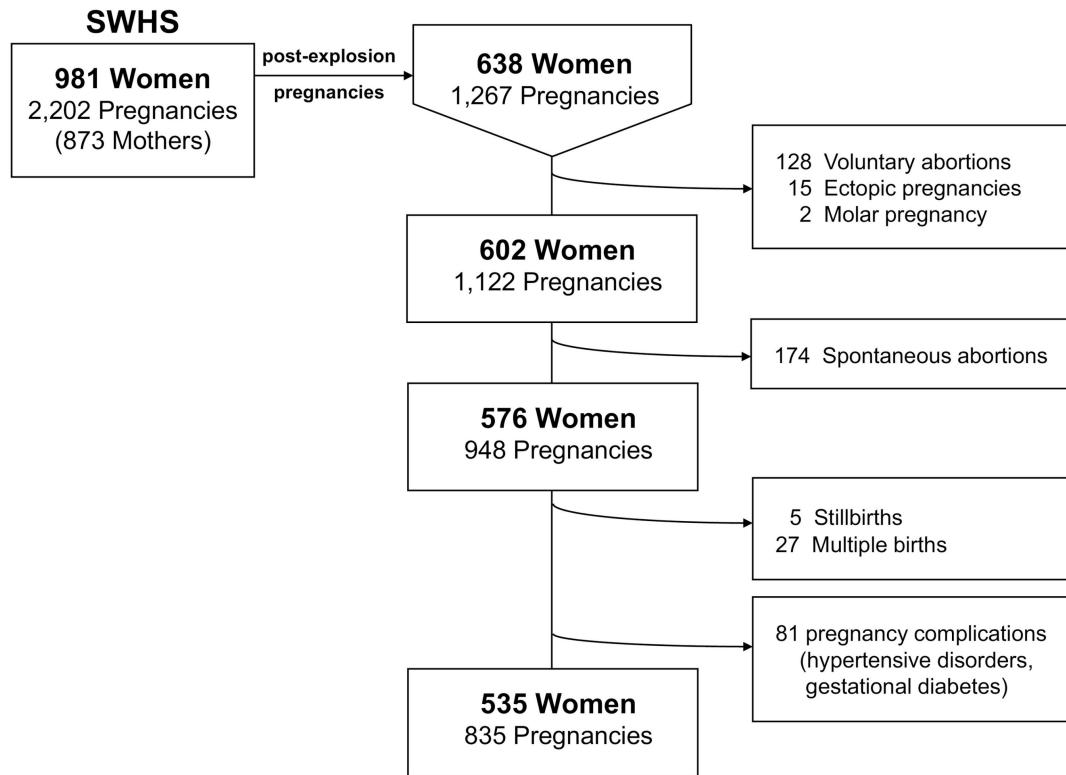


Figure 3. Post-explosion birth outcomes in the Seveso Women’s Health Study cohort, Seveso, Italy, 1976–2016

Table 1.

Select characteristics of cohort participants by follow-up study date, Seveso Women's Health Study, Seveso, Italy, 1976–2016

Characteristic	SWHS 1996–1998	Follow-up 2008–2009	Follow-up 2014–2016
	n (%)	n (%)	n (%)
Total	981 (100)	833 (84.9) ^{a,b}	705 (71.9) ^{a,c}
<u>Characteristics at explosion</u>			
Zone of residence			
A	167 (17.0)	142 (17.0)	123 (17.4)
B	814 (83.0)	691 (83.0)	582 (82.6)
Age (years)			
0–10	232 (23.7)	200 (24.0)	181 (25.7)
11–20	279 (28.4)	252 (30.3)	226 (32.1)
21–30	241 (24.6)	206 (24.7)	175 (24.8)
31–40	229 (23.3)	175 (21.0)	123 (17.4)
Menarche status			
Premenarche	284 (29.0)	247 (29.7)	221 (31.3)
Postmenarche	697 (71.0)	586 (70.3)	484 (68.7)
<u>Characteristics at interview</u>			
Age (years)			
mean (SD)	40.8 (11.7)	52.2 (11.1)	57.7 (11.0)
Range	21 – 63	32 – 73	38 – 80
Marital status			
Never	76 (7.7)	47 (5.6)	40 (5.7)
Ever	905 (92.3)	786 (94.4)	665 (94.3)
Maternal education			
Required	651 (66.4)	550 (66.0)	382 (55.4)
Secondary school	288 (29.4)	249 (29.9)	277 (40.2)
> Secondary school	42 (4.3)	34 (4.1)	30 (5.4)
Menopause status			
Premenopause	703 (71.7)	394 (47.3)	223 (31.6)
Postmenopause	278 (28.3)	439 (52.7)	482 (68.4)
Primary wage earner education ^d			
Required	627 (63.9)	529 (32.3)	376 (54.7)
Secondary school	354 (36.1)	252 (32.1)	311 (45.3)

^aPercent of initial cohort (n=981)

^b16 (1.6%) deceased, 36 (3.7%) could not be located or contacted, 96 (9.8%) refused

^c33 (3.3%) deceased, 64 (6.5%) could not be located or contacted, 179 (18.3%) refused

^dRequired (8 years of school), Secondary School (9–13 years), >Secondary School (>13 years)

Table 2.

Summary of health studies in Seveso residents.

Outcome	Author, Year	Study Population	Sample	Exposure Measure	Key Findings
<i>CHILD HEALTH</i>					
<i>Chloracne</i>	Caramaschi, 1981	Surveillance of children <14 years	146 cases, zones A, B, R 182 controls, zone nonABR	Zone	↑ % chloracne in Zone A
	Baccarelli, 2005	Case-control study	101 cases 211 controls	Plasma TCDD (1993–98)	↑ >10 ppt vs 10 ppt
<i>Liver Function</i>	Mocarelli, 1986	Surveillance 1976–1982	1,500 children 6–10 years, from zones A, B, R	Zone	↑ GGT (76–77) in Zone A boys ↑ ALT (76–77) in Zone A boys ↑ AST (80–82) in Zone A girls
	Ideo, 1982	Surveillance 08/76–12/76	31 children, from Zone A 6–8 years	Chloracne status	↑ d-glucuronic acid levels in children with chloracne
	Ideo, 1985	Surveillance of children 05/1981	34 zone A 61 zone B 121 zone R 61 zone nonABR	Zone	↔ d-glucuronic acid levels
	Mocarelli, 1991a	Surveillance 1976–1982	692 zone A 3,703 zone B 6,974 zone R 5,743 nonABR	Zone	↑ lymphocytes (76–77) in Zone A children 0–14 y ↔ lymphocyte response to PHA in Zone A children 0–14 y
<i>Tooth development</i>	Alaluusua, 2004	Dental exam 2001	48 zone A, B, R 65 nonABR	1976 serum TCDD	↑ developmental defects of enamel in children <5 years in 1976
<i>REPRODUCTIVE HEALTH</i>					
<i>Male fertility</i>	Mocarelli, 2008	Sperm quality study (1997–1998)	135 men, zone A, B, R and 1–26 y in 1976 184 men nonABR	1976 serum TCDD	1–9 y in 1976 ↓ sperm concentration ↓ motile sperm ↓ estradiol ↑ FSH 10–17 y in 1976 ↑ sperm concentration ↑ motile sperm ↓ estradiol ↑ FSH ↔ 17–26 years in 1976
	Eskenazi, 2010	SWHS (1996–1998)	463 women	1976 serum TCDD, TCDD estimated to pregnancy	↑ time to pregnancy ↑ infertility

Outcome	Author, Year	Study Population	Sample	Exposure Measure	Key Findings
<i>Menstrual cycle</i>	Baccarelli, 2005	Chloracne case-control study	45 cases 103 controls	Chloracne	↔ irregular cycle in cases vs controls
<i>Menstrual cycle</i>	Eskenazi, 2002b	SWHS (1996–1998)	301 women	1976 serum TCDD	↑ cycle length only in women who were premenarche in 1976
<i>Ovarian function</i>	Warner, 2007	SWHS (1996–1998)	363 women	1976 serum TCDD	↔ number or size of follicles ↔ ovulation ↔ progesterone, estradiol
<i>Endometriosis</i>	Eskenazi, 2002b	SWHS (1996–1998)	601 women	1976 serum TCDD	↑ endometriosis
<i>Fibromas</i>	Eskenazi, 2007	SWHS (1996–1998)	956 women	1976 serum TCDD	↔ fibroids
<i>Menopause</i>	Eskenazi, 2005	SWHS (1996–1998)	616 women	1976 serum TCDD	nonmonotonic ↑ early menopause 20.1–100 ppt ↑ early menopause > 100 ppt
CHRONIC HEALTH					
<i>Cancer</i>	Consonni, 2008	Cancer Mortality Study (1976–2001)	804 zone A 5,941 zone B 38,623 zone R 232,740 nonABR Total person-years: 6,192,864	Zone	↔ All cancer mortality (76–01) ↑ All cancer mortality after 20 years in Zone A (96–01) ↑ Lymphatic-hematopoietic cancer mortality in Zone A ↑ Lymphatic-hematopoietic cancer mortality in Zone B ↔ Lymphatic-hematopoietic cancer mortality in Zone R
	Pesatori, 2009	Cancer Incidence Study (1976–1996)	723 zone A 4,821 zone B 31,643 zone R 181,574 nonABR	Zone	↔ All cancer incidence (76–96) ↑ Breast cancer in Zone A (ns) ↑ Lymphatic-hematopoietic cancer in Zone A (ns) ↑ Lymphatic-hematopoietic cancer in Zone B
	Warner, 2002	SWHS (1976–1996)	981 women	1976 serum TCDD	↑ Breast cancer incidence
	Warner, 2011	SWHS (1976–2008)	833 women	1976 serum TCDD	↑ All cancer incidence
<i>Cardiovascular disease</i>	Consonni, 2008	Mortality Study (1976–2001)	804 zone A 5,941 zone B 38,623 zone R 232,740 nonABR	Zone	↔ All cause mortality ↑ IHD mortality in Zone A males ↑ Hypertension mortality in Zone A females
	Warner, 2014	SWHS (1976–2008)	965 women	1976 serum TCDD	↑ All CVD incidence (ns) ↑ IHD incidence (ns)
<i>Diabetes</i>	Consonni, 2008	Mortality Study (1976–2001)	804 zone A 5,941 zone B 38,623 zone R 232,740 nonABR	Zone	↑ Diabetes mortality in females in Zones A, B, R

Outcome	Author, Year	Study Population	Sample	Exposure Measure	Key Findings
<i>Bone health</i>	Warner, 2013	SWHS (1976–2008)	981 women	1976 serum TCDD	↔Diabetes ↔BMI, Obesity ↑Metabolic syndrome in <12y in 1976
	Eskenazi, 2014	SWHS (2008–2009)	340 women <20y in 1976	1976 serum TCDD	↔BMD (spine, total hip, femoral neck) ↑Bone structure in women exposed before peak bone mass
<i>Thyroid health</i>	Chevrier, 2014	SWHS (2008–2009)	909 women	1976 serum TCDD	↓TT4 in 1996 ↔ TSH, FT3, FT4 in 1996 ↓TT4 in 2008 ↔ TSH, FT3, FT4 in 2008
<i>Immune function</i>	Baccarelli, 2002	Immunology study (1992–1994)	62 zone A, B 58 zone nonABR	1992 plasma TCDD	→IgG w TCDD ↔IgM, IgA, C3, C4 w TCDD
<i>Neurologic function</i>	Pocchiarri, 1979	Surveillance 1977–1978	446 zone A, 255 zone B, R	Zone	↑ idiopathic clinical neurologic damage in Zone A
	Assenato, 1989	Surveillance 1985	193 chloracne cases		↔Abnormal electrophysiologic or nerve conduction velocity measures
	Ames, 2017a	SWHS (1996–1998) (2008–2009)	154 women 459 women	1976 serum TCDD	↔Physical function↔Working memory
SECOND GENERATION HEALTH					
<i>Sex ratio</i>	Mocarelli, 1996	Municipality birth registry	74 births (04/77 – 12/84)	Zone A	↓ Sex ratio (1977–84) ↔Sex ratio (1985–94)
	Mocarelli, 2000	Municipality birth registry	674 births (04/77 – 12/96)	1976 serum TCDD	↓Sex ratio in fathers < 19 years in 1976 and TCDD >15 ppt ↔Sex ratio in fathers > 19 years in 1976 and TCDD >15 ppt
<i>Neonatal thyroid</i>	Baccarelli, 2008	Neonatal Screening Registry (1994–2005)	56 zone A 425 zone B 533 nonABR	Zone	↑b-TSH in Zone A ↑b-TSH in Zone B ↑b-TSH>5μU/mL in Zone A
<i>Male fertility</i>	Mocarelli, 2011	Chloracne Study	51 mother-child pairs	TCDD extrapolated to pregnancy	↑b-TSH w TCDD and TEQ extrapolated to pregnancy
		Sperm quality study (2002–2003)	39 Seveso sons born 1977–1984 58 unexposed male blood donors	lactational exposure	↓sperm concentration ↓progressive motility ↓FSH

Table 3.

Results of relative risk regression models^a showing the relationship between maternal TCDD exposure and spontaneous abortion, SWHS, Seveso, Italy, 1976–2016.

Exposure	Post-explosion pregnancies	Spontaneous abortion	
		n / Total ^d	Adj.RR (95% CI) ^{b, c}
1976 Serum TCDD	All	174/ 1,122	0.85 (0.69, 1.05)
	First	79/ 573	0.86 (0.63, 1.17)
Estimated TCDD at Pregnancy	All	174/ 1,122	0.90 (0.72, 1.12)
	First	79/ 573	0.79 (0.56, 1.13)
Estimated TEQ at Pregnancy	All	69/ 344	1.27 (0.50, 3.23)
	First	21/ 118	1.13 (0.32, 4.05)

^aResults are for a 10-fold increase in serum TCDD or TEQ.

^bAdjusted for maternal height, pre-explosion history of spontaneous abortion, parity, and maternal age.

^cIn TEQ model, pre-explosion history of spontaneous abortion and parity are omitted by collinearity.

^dTotal excludes ectopic pregnancies (n=15), molar pregnancies (n=2), and voluntary abortions (n=128). However, first pregnancy is defined as the first pregnancy out of the 1,267 postexplosion pregnancies.

Table 4.

Sociodemographic and pregnancy characteristics by initial maternal 1976 serum TCDD levels for 574 women with 943 post-explosion live births, SWHS, Seveso, Italy, 1976–2014.

Characteristic	N (%)	Initial 1976 Maternal Serum TCDD (ppt) Median (IQR)
Total Women	574 (100.0)	61.2 (28.6, 162.0)
Zone of residence *		
A	100 (17.4%)	264.5 (102.1, 919.0)
B	474 (82.6%)	49.7 (24.7, 111.0)
Age at explosion (years) *		
0–10	166 (28.9)	157.5 (52.9, 322.0)
11–20	241 (42.0)	52.8 (25.4, 102)
21–30	145 (25.3)	40.6 (22, 77.0)
31–40	22 (3.8)	41.5 (27.7, 110)
Menarche status at explosion *		
Premenarche	213 (37.1)	131.0 (50.6, 286.0)
Postmenarche	361 (62.9)	44.4 (22.5, 86.7)
Pre-explosion parity *		
0	458 (79.8)	70.6 (31.4, 191.0)
1	74 (12.9)	36.6 (21.6, 70.4)
2	42 (7.3)	35.7 (25.1, 70.3)
Total livebirths	943 (100.0)	60.2 (27.2, 153.0)
Age at pregnancy (years) *		
<25	157 (16.7)	45.1 (21.3, 102.0)
25–39	320 (33.9)	54.7 (27.2, 125.0)
30–34	281 (29.8)	66.2 (27.7, 176.0)
35	185 (19.6)	64.1 (33.2, 180.0)
Smoking during pregnancy *		
No	850 (90.1)	61.1 (28.6, 163.0)
Yes	93 (9.9)	45.4 (20.7, 98.3)
Weight gain during pregnancy (kg) ^a		
<10	197 (20.9)	67.5 (36.6, 141.0)
10–14	437 (46.3)	53.7 (26.3, 136.0)
15–19	179 (19.0)	62.8 (22.1, 223.0)
20	103 (10.9)	63.2 (28.8, 153.0)
Infant sex		
Male	490 (52.0)	54.3 (26.7, 129.0)
Female	453 (48.0)	64.4 (28.8, 176.0)

* Anova p-value <0.05

^aMissing data (n=26)

Table 5.

Results of linear and logistic regression models^a showing the relationship between maternal TCDD exposure and length of gestation and fetal growth, SWHS, Seveso, Italy, 1976–2014.

Exposure	Pregnancies	Gestational age (weeks)		Preterm		Birthweight (grams)		Small-for-Gestational-Age	
		N	Adj. β (95% CI) ^b	n/Total	Adj. OR (95% CI) ^b	n	Adj. β (95% CI)	n/Total	Adj. β (95% CI)
1976 Serum	All	834	-0.37 (-1.84, 1.10)	47/834	1.18 (0.71, 1.94)	834	-38.2 (-100.2, 23.7) ^c	74/834	1.33 (0.92, 1.94) ^c
TCDD	First	516	-0.86 (-2.61, 0.90)	32/516	1.17 (0.66, 2.08)	516	-60.5 (-130.4, 9.5) ^c	52/516	1.39 (0.90, 2.15) ^c
TCDD at	All	834	0.94 (-0.73, 2.60)	47/834	0.68 (0.35, 1.36)	834	28.4 (-41.7, 98.5) ^d	74/834	1.21 (0.75, 1.94) ^d
Pregnancy	First	516	0.44 (-1.42, 2.30)	32/516	0.69 (0.33, 1.45)	516	-5.5 (-87.5, 76.6) ^d	52/516	1.40 (0.82, 2.37) ^d
TEQ at	All	228	9.6 (3.3, 15.8)	17/228	0.09 (0.01, 1.06)	228	235.1 (-17.6, 487.7) ^d	15/228	1.53 (0.10, 22.85) ^d
Pregnancy	First	113	8.5 (-1.8, 18.8)	12/113	0.10 (0.01, 1.68)	113	295.7 (-102.3, 693.8) ^d	7/113	0.96 (0.01, 154.2) ^d

^aResults are for a 10-fold increase in serum TCDD or TEQ.

^bAdjusted for maternal height, pre-explosion history of low birthweight, year of pregnancy, parity, and maternal age.

^cAdjusted for maternal height, pre-explosion history of low birthweight, year of pregnancy, parity, maternal age, maternal smoking.

^dAdjusted for maternal height, pre-explosion history of low birthweight, year of pregnancy, parity, maternal age, maternal smoking, gestational weight gain.

Table 6.

Summary of pregnancy outcomes of SWHS daughters and sons, Seveso Second Generation Study, Seveso, Italy, 2014–2016.

	Daughters (n=76) n (%)	Sons (n=45) n (%)
Total pregnancies	134 (100.0)	76 (100.0)
Currently pregnant	0 (0.0)	7 (9.2)
Live birth ^a	96 (71.6)	53 (69.7)
Spontaneous abortion	32 (23.9)	10 (13.2)
Voluntary abortion	4 (3.0)	3 (4.0)
Ectopic pregnancy	2 (1.5)	1 (1.3)
Stillbirth	0 (0.0)	1 (1.3)
Unknown	0 (0.0)	1 (1.3)
Live birth ^a	96	53
Birthweight (grams) ^b	3,188 (±560)	---
Preterm birth (<37 weeks) ^c	9 (8.6)	5 (9.4)
Low birthweight (<2,500 grams) ^c	7 (6.8)	7 (13.2)

^aLive births include: 94 singletons and 2 twins (daughters); 49 singletons and 4 twins (sons).

^bmean (± standard deviation).

^cn (%) of live births.