Dioxin exposure and non-malignant health effects: a mortality study

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Abstract

***Objective*—To investigate, in a population heavily exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the possible unusual occurrence of diseases other than cancer.**

***Methods*—Five year extension of the follow up of the cohort involved in the Seveso accident. Soil measurements identified three exposure zones: (A) highest contamination, (B) substantial, and (R) low but higher than background contamination. Blood TCDD measurements, although limited in number, confirmed zone exposure ranking. The 15 year mortality in the exposed cohort was compared with that of a large population in the surrounding non-contaminated territory. Relative risks (RRs) and 95% confidence intervals (95% CIs) were estimated with Poisson regression techniques.**

***Results*—The already noted increased occurrence of cardiovascular deaths was confirmed, in particular in zone A, among males for chronic ischaemic heart disease (five deaths, RR 3.0, 95% CI 1.2 to 7.3), and among females for hypertensive disease (three deaths, RR 3.6, 95% CI 1.2 to 11.4) and chronic rheumatic heart disease. Novel findings were the increase of chronic obstructive pulmonary disease, most notably among males in zone A (four deaths, RR 3.7, 95% CI 1.4 to 9.9) and females in zone B (seven deaths, RR 2.4, 95% CI 1.1 to 5.1); and from diabetes, which was significantly increased in females in zone B (13 deaths, RR 1.9, 95% CI 1.1 to 3.2). In zone R, chronic ischaemic heart disease (males and females), hypertension (females), and diabetes (females) showed less pronounced, although significant excesses.**

***Conclusions*—As well as high TCDD exposure, the accident caused a severe burden of strain in the population. Both these factors might have contributed to the noted increased risks (in particular, circulatory and respiratory). The cardiovascular and immune toxicity of TCDD, as well as its complex interaction with the endocrine system, might be relevant to the explanations of these findings. These results, although not conclusive, concur with previous data in suggesting cardiopulmonary and endocrine effects in humans highly exposed to TCDD.**

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The public health concern surrounding polychlorinated dibenzo-para-dioxins (PCDDs) is justified by the extreme toxicity of some of these compounds, by their ubiquity in food, and their persistence in the environment.[1 2] The most toxic congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), showed a wide range of severe effects in animal experiments, including immunotoxicity, developmental and reproductive toxicity, teratogenicity, and carcinogenicity.[3] Effects in humans have been extensively studied, but the picture is not fully consistent and is probably incomplete. As well as cancer,[4 5] effects on skin,[6–14] liver,[10 15–21] thyroid,[22 23] immune system,[24] lipid[16 21 25] and glucose metabolism,[21 26] and the circulatory system have been reported.[27 28] The possible association of reproductive and developmental effects with TCDD exposure is still controversial.[29]

One of the populations most heavily exposed to TCDD is that involved in the Seveso, Italy accident that occurred in 1976 in a chemical plant where 2,4,5 trichlorophenol was manufactured. The accident originated with an uncontrolled exothermic reaction in one of the production kettles, causing a sudden surge in temperature and pressure that blew a safety device. The reactor content, a fluid mixture containing some 2900 kg of organic matter, as well as sodium hydroxide, ethylene glycol, sodium trichlorophenate, trichlorophenol, and a substantial amount of 2,3,7,8,-TCDD, was released into the air through an exhaust pipe and deposited as far as 6 km south east of the factory.

The most heavily contaminated area (zone A) covered some 87 hectares, and mean concentrations of 2,3,7,8,-TCDD between 15.5 μg/m^2 and 580.4 μg/m^2 were recovered from soil samples.[30] A further contamination zone with concentrations not exceeding 50 μg/m^2 covered 270 hectares (zone B). A third area of 1430 hectares extended to where detectable levels of TCDD (nominally, <0.75 μg/m^2) were measured. This zone R had a patchy, low contamination generally below 5 μg/m^2. After a few weeks, most of the residents in zone A (over 700 people) were ordered to leave the area. In zone B, people remained in their houses but strict regulations were issued to avoid consumption of home grown products (vegetables and animals). Limiting regulations were also issued for residents of zone R.

In the early period after the accident, the most obvious effect was the extremely high occurrence of chloracne (a skin lesion known to be associated with exposure to chlorinated

polyaromatic hydrocarbons)[31] that affected 19.6% of children aged 3–14 years living in zone A. Several surveys on early and mid-term health outcomes possibly associated with exposure to TCDD—for example, neurological, immunological, and hepatic impairment, cytogenetic effects, birth defects, and abortion rates—were conducted under the supervision of an international steering committee.[32 33] Most of these investigations yielded inconclusive results, due in part to flaws in study planning—for example, the lack of appropriate reference groups—and especially to the complex crisis situation that undermined the implementation of a sound epidemiological design and jeopardised essential study steps—for example, standardisation of methods and procedures and subjects' compliance.[32 34] In 1984 the steering committee concluded that "it is obvious that no clear-cut adverse health effects attributable to TCDD, besides chloracne, have been observed", but indicated the need for further studies embracing the time span of appearance of possible late effects.[35]

Long term investigations on cancer incidence and mortality were designed and implemented. In the first decade after the accident, the incidence study showed an increase for cancer of the hepatobiliary tract, for lymphatic and haemopoietic neoplasms, and for soft tissue sarcoma.[36] For this study the time has been extended. The mortality study also examined non-cancer causes,[37] and we report here updated results for a 15 year period, July 1976 to June 1991.

Materials and methods

The accident area is part of a specific region north of Milan (named Brianza), and it included part of the territory of two health districts encompassing 11 municipalities. The study population comprised all people of any age and both sexes residing in one of those municipalities at the date of the accident. The information about town and street address at the date of the accident was used to attribute subjects to the exposure category corresponding to one of the contamination zones, or to the surrounding non-contaminated territory the population of which (nearly sixfold larger than the exposed population, table 1) was adopted as a source of reference data. The 1981 census data for the Province of Milan were examined to compare the characteristics of the contaminated municipalities with the reference area for occupational, social, and educational background.[38] No differences were found in educational level achieved, economic sector of employment, and position at work, family size, and housing (number of rooms and available services). Also, the population of all municipalities within the two joint health districts shared the same hospitals, health services, and family physicians.

Exposure classification was based on environmental contamination data—namely, results of TCDD measurements in soil. Within five weeks of the accident, the contaminated area was subdivided into zones A, B, and R in descending amount of exposure. The total amount of TCDD released is considered to be in the order of several kilograms.[33] The actual level of exposure in each zone can be evaluated in comparison with the blood values of TCDD measured in subsamples of the study population. In blood samples taken in 1976–7, the following blood median concentrations were measured among the most exposed subjects older than 13 years: zone A 177 subjects, 443 pg/g blood lipids; zone B 54 subjects, 87 pg/g blood lipids; zone R 17 subjects, 15 pg/g blood lipids.[4] In 1992–3, additional samples were collected in randomly selected subjects older than 20 years participating in an ongoing molecular epidemiology study. The TCDD blood concentrations (geometric mean), nearly 20 years after the accident, were: zone A seven subjects, 53.2 pg/g blood lipids; zone B 51 subjects, 11.0 pg/g blood lipids; reference area 55 subjects, 4.9 pg/g blood lipids.[39]

Table 1 Mortality, 1976–91, from non-malignant causes in the Seveso population (study population)

Zones	Subjects (n)	Not traced (n (%))	Males (person-years)	Females (person-years)
A	805	7 (0.9)	5541	5975
B	5943	53 (0.9)	42219	41391
R	38625	361 (0.9)	265408	271483
Reference	232747	2066 (0.9)	1536724	1622631

Each person in the population was followed up from the date of the accident to the end of 1991. For those who left their initial residence, the search started from the last known address, from the information recorded on municipal vital statistics registries, which are available throughout the whole country, until the person was located and the vital status ascertained. Exposed and control subjects were traced concurrently and with the same methods. All tracing procedures were implemented without knowledge of the subjects' exposure status. This assured, among other things, a non-differential accuracy in the ascertainment and coding of cause of death.

The stability of the mortalities derived from the control population was evaluated through a comparison with the rates of the population of the entire region of Lombardy (nearly 9 000 000 inhabitants).[37] Minor discrepancies emerged. The temporal trend of mortality in the area was also examined and, for one cause of cancer, mortality was higher in the TCDD contaminated zones than in the reference area, even before the accident.[40] Comparison of rates was performed with standard Poisson regression techniques,[41] controlling for age and calendar period. Relative risks (RRs) and 95% confidence intervals (95% CIs) were estimated, as well as the number of expected deaths for each cause of death. Separate analyses were performed by zone, sex and duration of follow up, which in this particular analysis, corresponded with the number of years elapsed since initial exposure (latency).

Results

Table 1 shows the results of the follow up. Ascertainment of vital status was successful for over 99% of the whole cohort, and the success rate did not vary across zones.

Table 2 Mortality, 1976–91, from non-malignant causes in the Seveso population (males, all ages)

Cause of death	Zone A			Zone B			Zone R		
ICD-9	Observed deaths	Relative risk	95% CI	Observed deaths	Relative risk	95% CI	Observed deaths	Relative risk	95% CI
All causes (1-999)	39	1.0	0.8 to 1.4	255	0.9	0.8 to 1.1	1898	1.0	1.0 to 1.1
Infectious, parasitic disease (1-139)	(0.2)			(1.2)			8	1.0	0.5 to 2.1
Diabetes (250)	(0.6)			6	1.3	0.6 to 2.9	37	1.1	0.8 to 1.6
All circulatory disease (390-459)	21	1.6	1.1 to 2.5	90	0.9	0.7 to 1.1	719	1.1	1.0 to 1.2
Hypertension (400-405)	1	2.3	0.3 to 16.5	(3.7)			32	1.3	0.9 to 1.9
Ischaemic heart disease (410-414)	9	1.5	0.8 to 2.9	36	0.8	0.6 to 1.2	316	1.1	0.9 to 1.2
Myocardial infarction (410)	4	0.9	0.3 to 2.5	17	0.6	0.4 to 0.9	188	0.9	0.8 to 1.1
Chronic ischaemic heart disease (412,414)	5	3.0	1.2 to 7.3	18	1.3	0.8 to 2.1	126	1.4	1.1 to 1.7
Cerebrovascular disease (430-438)	5	1.5	0.6 to 3.7	30	1.2	0.8 to 1.7	190	1.1	0.9 to 1.3
Respiratory disease (460-519)	5	2.4	1.0 to 5.7	13	0.7	0.4 to 1.3	122	1.0	0.9 to 1.3
Chronic obstructive pulmonary disease (490-493)	4	3.7	1.4 to 9.9	9	1.0	0.5 to 1.9	67	1.1	0.8 to 1.4
Digestive disease (520-579)	2	0.6	0.2 to 2.6	15	0.7	0.4 to 1.2	155	1.1	0.9 to 1.3
Cirrhosis of liver (571)	2	0.9	0.2 to 3.6	12	0.8	0.5 to 1.5	110	1.1	0.9 to 1.3
Accidents (800-999)	2	0.7	0.2 to 2.9	20	1.0	0.6 to 1.6	125	1.0	0.8 to 1.2

( )=Number of expected deaths when observed=0.

Table 2 shows the mortality among males. In all zones, overall mortality did not differ from expectations. In zone A, a borderline significantly increased mortality from circulatory and respiratory diseases was found. Mortality from chronic ischaemic heart disease was notably high, with a threefold increase; four out of five deaths occurred in the first decade after the accident (RR 3.5; 95% CI 1.3 to 9.3). Mortality from cerebrovascular disease was only slightly, non-significantly increased, and the increased risk was confined to the first decade after the accident (five deaths; RR 2.2; 95% CI 0.9 to 5.4). The excess mortality from respiratory disease, mainly chronic obstructive pulmonary diseases, had an RR approaching 4.0. The risk was higher in the first five years after the accident, with three deaths and RR 7.1 (95% CI 2.3 to 22.4). Mortality from digestive disease and accidents did not depart from expectations. In zone B, diabetes, chronic ischaemic heart disease, and cerebrovascular disease showed a 20%–30% non-significant excess. Deaths due to myocardial infarction were fewer than expected. In zone R, departures from expectations were found for chronic ischaemic heart disease, and possibly for hypertension.

Table 3 shows mortality among females. Overall mortality was not increased. In zone A, significantly increased risks were found for chronic rheumatic heart disease and hypertensive vascular disease; a possibly increased mortality from diabetes, based on two deaths, was also found. In zone B, mortality from diabetes showed a significant 90% excess. Nine out of 13 deaths from diabetes occurred in the second decade after the accident giving an RR of 3.1 (95% CI 1.6 to 6.1). A significantly increased risk was also found for chronic obstructive pulmonary diseases. In zone R, mortality from hypertension was clearly increased; diabetes, chronic ischaemic heart disease, cerebrovascular disease, and chronic obstructive lung disease showed slightly increased risks of borderline significance.

Discussion

In the 15 year period after the Seveso accident, increased deaths from cardiovascular disease, chronic obstructive pulmonary disease, and diabetes were found in the population residing at the time of the accident in the area contaminated by TCDD.

The cardiovascular mortality was most prominently increased in the area of highest exposure and among males. Chronic ischaemic heart disease, in particular, was significantly increased in zone A (with most of the deaths occurring within 10 years), and increased, although to a lesser extent, in zones B and R. Among females, no overall increase in cardio-

Table 3 Mortality, 1976-91, from non-malignant causes in the Seveso population (females, all ages)

Cause of death	Zone A			Zone B			Zone R		
ICD-9	Observed deaths	Relative risk	95% CI	Observed deaths	Relative risk	95% CI	Observed deaths	Relative risk	95% CI
All causes (1-999)	30	1.0	0.7 to 1.5	176	1.0	0.8 to 1.1	1589	1.0	1.0 to 1.1
Infectious, parasitic disease (1-139)	(0.1)			(0.5)			4	1.1	0.4 to 3.1
Diabetes (250)	2	1.8	0.4 to 7.3	13	1.9	1.1 to 3.2	74	1.2	1.0 to 1.6
All circulatory disease (390-459)	12	1.0	0.6 to 1.7	74	1.0	0.8 to 1.2	759	1.1	1.0 to 1.2
Chronic rheumatic heart disease (390-398)	3	15.8	4.9 to 50.4	(1.2)			11	1.2	0.6 to 2.3
Hypertension (400-405)	3	3.6	1.2 to 11.4	3	0.6	0.2 to 1.8	72	1.6	1.2 to 2.0
Ischaemic heart disease (410-414)	1	0.3	0.0 to 2.0	24	1.1	0.7 to 1.6	210	1.0	0.9 to 1.2
Myocardial infarction (410)	1	0.6	0.1 to 4.0	9	0.8	0.4 to 1.6	77	0.8	0.6 to 1.0
Chronic ischaemic heart disease (412,414)	(1.9)			15	1.3	0.8 to 2.2	133	1.3	1.0 to 1.5
Cerebrovascular disease (430-438)	2	0.5	0.1 to 2.0	26	1.0	0.7 to 1.5	258	1.2	1.0 to 1.3
Respiratory disease (460-519)	2	1.3	0.3 to 5.3	8	0.9	0.4 to 1.7	71	0.8	0.7 to 1.1
Chronic obstructive pulmonary disease (490-493)	1	2.1	0.3 to 14.9	7	2.4	1.1 to 5.1	34	1.3	0.9 to 1.9
Digestive disease (520-579)	2	1.2	0.3 to 5.0	13	1.3	0.8 to 2.3	88	1.1	0.9 to 1.4
Cirrhosis of liver (571)	(0.9)			4	0.7	0.3 to 1.9	46	1.0	0.8 to 1.4
Accidents (800-999)	2	1.5	0.4 to 6.0	7	0.8	0.4 to 1.8	69	1.0	0.8 to 1.4

( )=Number of expected deaths when observed=0.

vascular mortality was found. Deaths from chronic rheumatic heart disease and hypertension, although few in number, showed a remarkable excess in zone A and a lower increase in zone R, whereas chronic ischaemic heart disease was slightly in excess in zones B and R.

The excessive mortality from respiratory disease among males was clearly confined to zone A and was due to chronic obstructive pulmonary disease. Mortality from this disease was also increased among females, particularly in zones A and B.

Mortality from diabetes was increased among females in all zones, and significantly so in zone B. Among males, no deaths from diabetes were found in zone A, and the increase in other zones was only suggestive.

The varying population size in different zones had a major influence on the power of the study to detect unusual relative risks. The small number of deaths in some of the subcohorts (especially in zone A and in subcohorts obtained after stratification by—for example, duration of exposure or latency) still limits interpretation of the results.

Exposure definition solely based on levels of soil contamination is another limitation. However, results of measurements of TCDD in blood, although limited,[4 39] support the plausibility of the adopted exposure classification. Inhabitants of zone A had the highest exposure; this was able to be documented because of the long half life of TCDD, and could be shown many years after they had left the area. In zone B, exposure was high and people living in the area might have been accumulating high doses over time. Biological data also confirmed that in zone R, the least contaminated zone, exposure was low, but definitely higher than background levels. Measured concentrations in a sample of the reference population were, as expected,[39] very close to the estimated background levels in industrial countries.[42]

The comparability of the relevant features of the exposed and reference populations was verified on the basis of the census data and not by comparing individual people's characteristics. Despite this limitation, differences in health determinants—such as those necessary to explain the noted differential mortality from certain causes—would hardly have gone undetected.

The increased risk of cardiovascular disease in the exposed population was from chronic ischaemic heart disease, chronic rheumatic heart disease, and hypertension.

The effects of TCDD on the cardiovascular system have been examined in several animal species, showing that TCDD can alter cardiac function and morphology. Reported effects include functional disturbances,[43–46] preatherosclerotic lesions in the aorta,[45] myocardial degeneration,[47] ventricular dilatation, and myocardial hypertrophy.[48] Experimental data also showed that TCDD causes increased serum triglycerides and cholesterol,[49–51] well known risk factors for cardiovascular diseases.[52]

Epidemiological studies in human populations yielded inconsistent findings. Cross sectional medical studies could not detect differences between exposed and non-exposed workers.[18 53 54] Participation bias and lack of clinically verified diagnoses affect the validity of these results. The largest occupational mortality studies in workers exposed to chlorophenoxy herbicides or chlorophenols contaminated with TCDD, either in Europe or in the United States, did not detect an increased risk of cardiovascular diseases.[55 56] The main focus of these studies was, however, on cancer mortality; analysis by specific categories of cardiovascular deaths was not reported; and detailed evaluation of exposure relative to cardiovascular outcomes was lacking. Moreover, the RR estimates were around 1.00, whereas when the mortality of an occupational cohort is compared with that of the general population, a deficit of cardiovascular mortality is usually found.[57] The absence of the so called "healthy worker effect" might actually suggest that even in those industrial cohorts exposed to TCDD an increased cardiovascular mortality existed. Three occupational studies were able to investigate mortality from ischaemic heart disease relative to estimated individual TCDD doses. One did not detect an effect of TCDD on mortality from ischaemic heart disease,[58] whereas two showed a positive relation: mortality from ischaemic heart disease increased with increasing TCDD concentrations.[27 28]

Mortality and morbidity due to circulatory disease have been examined also among Vietnam veterans. The results of the various studies are not consistent. Often they refer to self reported diagnosis; in some instances control of confounders is lacking; combat experience itself has been reported as a possible causal factor; no clear dose response emerged when examined.[59] Only one study reported an increased mortality from circulatory disease among "non-flying enlisted personnel", a subgroup of "ranch hands" (troops employed in the aerial spraying of "agent orange") with higher current TCDD serum concentrations than other ranch hands.[60]

In the Seveso population, two components of the accident experience emerge as possible explanations of the excesses found: the chemical exposure and the disaster experience with its burden of psychosocial stressors. For chronic ischaemic heart disease, the role of accident stressors was most probable in exacerbating already existing ill health. This hypothesis would be consistent with the highest risks detected in zone A, where subjects experienced the greatest psychosocial impact of the accident—for example, many had to leave their houses and family run shops—social support was thought to be insufficient and the experience of rejection was common; great uncertainty was experienced about the future, including health of children and future generations.[61 62] The hypothesis would also be consistent with the early occurrence of excess deaths after the accident and with the prevailing type (chronic) of cardiovascular disorders. The role of TCDD exposure as a possible risk factor is consistent with the zone A population being

the most severely affected, and is supported by experimental and epidemiological data. The increased mortality from hypertension might be explained by these same risk factors. The increase of rheumatic heart disease might instead be related, at least by way of hypothesis, to the immunotoxic properties of TCDD. This, however, was one of the few causes of death for which the reference population was found to be probably underestimated.[37]

The excess mortality from respiratory diseases was mainly from chronic obstructive pulmonary disease (COPD), mainly in zone A males and zone B females. No clear relation with time since first exposure emerged among females; whereas the excess found among zone A males was mainly detected in the first five years after the accident, and mainly affected elderly men. One reasonable hypothesis is that the same accident related factors discussed for cardiovascular deaths could have precipitated early deaths among people with pre-existing chronic respiratory disease. Another possible explanation rests with a differential death certification in exposed and non-exposed areas, given the higher than usual proportion of COPD where an excess of deaths related to the respiratory system was found; this explanation seems less probable because there was a common certifying physician, and because COPD was not considered to be an illness related to TCDD. Coding, on the other hand, was performed by the same person unaware of the exposure status of the subjects. Previous cohort and cross sectional studies in humans do not support an association between TCDD exposure and non-malignant respiratory disorders.[63] The immunotoxic action of TCDD might be a relevant and plausible explanatory factor: impaired protection and defence against episodes of respiratory infection play a major part in the natural history of chronic obstructive pulmonary disease.[64]

Smoking is supposed to be considered as the main confounding factor for the increase of both cardiovascular and respiratory diseases. All the available evidence is, however, against this explanation, including the absence of increased risks from respiratory and other cancers associated with smoking[65]; the similar social, cultural, and occupational characteristics (all variables possibly related to personal habits) of the index and control population; the highest relative risks in the most exposed subgroups; and the extremely large differences in smoking habits needed to explain confounding relative risks as high as those found.[66]

Diabetes mellitus was increased in zones A and B among females. The risk was particularly high in the second decade after the accident. Recent investigations in male populations exposed to dioxin support a positive association between TCDD serum concentrations and diabetes and fasting serum glucose concentrations.[21 67 68] In Seveso, however, only women were affected. This finding might be explained by the complex, and not fully understood, interaction of dioxin with hormonal factors,[69] or the systematically higher TCDD concentrations in females than in males.[39] It was unlikely that diet played any part. On the contrary, diet is one of the main characteristics by which the area including the exposed and reference territory is identified as a particular regional entity.

In conclusion, the study of non-malignant health effects in this population accidentally exposed to substantial amounts of relatively pure TCDD uncovered an increased mortality from cardiovascular and respiratory diseases and from diabetes, which could not be explained solely by chance, bias, or confounding. Although no definite conclusion can be drawn at this stage, the association of the increased risks with exposure to TCDD seems possible and plausible.

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