ELEVATED DIOXIN LEVELS IN CHLORACNE CASES TWENTY YEARS AFTER THE SEVESO, ITALY ACCIDENT

Andrea Baccarelli[1], Angela C Pesatori[1], Dario Consonni[1], Paolo Mocarelli[2], Donald G Jr. Patterson[3], Matteo Bonzini[1], Sara M Giacomini[1], Neil E Caporaso[4], Pier Alberto Bertazzi[1], Maria Teresa Landi[4]

[1]EPOCA Research Center, University of Milan, Milan, Italy
[2]Department of Laboratory Medicine, University of Milan-Bicocca, Desio, Italy
[3]Centers for Disease Control and Prevention, Atlanta, GA, USA
[4]Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda, MD, USA

Introduction

In July 1976, an industrial accident contaminated a residential area surrounding Seveso, Italy, with high levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD).[1-3] The exposure was acute, relatively pure, and affected more than 45,000 men, women, and children. By February 1978, 193 chloracne cases, mostly children, had been identified in the exposed population.[4, 5] Twenty years after, we conducted a case-control study on subjects diagnosed with chloracne and control subjects, who had not developed chloracne after the accident, to evaluate their TCDD plasma levels, as well as the exposure-response relationship and possible determinants of susceptibility to TCDD effects in this population.

Methods and Materials

The design of the study and subjects recruitment have been described previously.[5] Briefly, Between January 1993 and April 1998, we recruited 101 well-documented, previously diagnosed chloracne cases (56 males, 45 females), who had been routinely followed up since the accident, and 211 controls (108 males, 103 females) from the same area. Chloracne diagnoses were made using standard criteria approved by the EEC commission, based on the type of lesions (comedones, cysts, pustules) and their distribution (face, neck, chest, back, other).[6] A questionnaire including data on demographics, lifestyle, foods consumed at the time of the accident, residential history, and occupations was administered by trained interviewers, who also determined individual pigmentary characteristics. The health status of the study subjects, as well as of their children born after the date of the accident, was ascertained by collecting information on an extensive list of diseases, medication use, and reproductive history. We obtained written informed consent from all participants. The local Institutional Review Board reviewed and approved the study. Plasma TCDD was measured at the Centers for Disease Control and Prevention (CDC) using a high-resolution gas chromatographic/high-resolution mass spectrometric analysis performed on human

plasma.[7] Plasma samples from 3 cases (3.0%) and 16 controls (7.6%) were inadequate for the assay and were excluded from the statistical analyses based on plasma TCDD. Plasma TCDD levels were below detection limit in 163 (55.6%) of the 293 subjects with samples adequate for the dioxin assay. Among them, all but ten subjects had detection limit below 10 ppt, lipid adjusted (equivalent to 10 pg g^{-1} fat). We estimated the dioxin levels for these ten subjects by dividing their detection limit by 2.[8] Subsequently, we divided plasma TCDD in two categories <=10 ppt or >10 ppt. The cut-off of 10 ppt is commonly considered to separate background from elevated TCDD plasma levels.[9-12] In univariate analysis, we used the Fisher's exact test to assess significance of differences between proportions. We calculate Odds ratios (ORs), 95% confidence intervals (CIs) and tests for trend by means of unconditional logistic regression models, adjusted by age, gender, or by age, gender, and zone of residence to take into account the sampling proportions. We performed all analyses using Stata 8.0. All reported *p* values are two-sided.

Results and Discussion

Current plasma TCDD ranged from background levels to 475.0 ppt and was higher than 10 ppt in seventy-eight (26.6%) of the 293 subjects with adequate plasma samples. Proximity of primary residence to the site of the accident was a strong determinant of elevated TCDD levels (Table 1). Relative to the individuals in the non-contaminated area, subjects who lived in A zone at the time of the accident were approximately 66 times more likely to have elevated plasma TCDD (OR=65.9, 95% CI: 16.6-262.1 for TCDD>10 ppt), while those in B and R zone had ORs of 25.8 (95% CI: 7.0-95.0) and 3.2 (95% CI: 0.5-18.4), respectively ($p<0.001$ for trend across zone categories). Plasma TCDD exhibited a nearly linear trend by age. Elevated TCDD levels were approximately 6 times more frequent (OR=5.9; 95% CI 2.3-15.1) in subjects older than 18 years at the time of the accident relative to individuals younger than 8 years ($p<0.001$ for trend across age categories). As expected,[13] women had higher plasma TCDD levels than men (OR=5.3, 95% CI: 2.5-11.2). Occurrence of elevated TCDD levels increased with increasing BMI (OR=1.6, 95% CI: 0.6-4.0 for BMI 21.7-25 kg/m^2 and OR=2.8, 95% CI: 1.0-4.0 for BMI>25 kg/m^2, relative to BMI<21.7 kg/m^2; $p=0.04$ for trend across BMI categories). All statistical models were adjusted for age, gender, and zone of residence. We evaluated whether personal behaviour and events at the time of the accident were associated with elevated dioxin levels. Lower plasma TCDD levels were found among residents of the study area who reported to have been on vacation at the date of the accident (OR=0.1, 95% CI: 0.03-0.7 for plasma TCDD>10 ppt). Subjects who had eaten home-grown poultry or livestock had higher odds of elevated plasma TCDD (OR=4.9; 95% CI: 2.0-11.7). Similarly, subjects who have had direct experience of the accident (i.e., saw or smelt the toxic cloud, heard the explosion, experienced eye/throat irritation or itching of the skin), and those who recalled the details of the accident (e.g., they could correctly remember the date and/or the time of the accident) had higher TCDD plasma levels in the analyses adjusted by age and gender. However, this association was not statistically significant when we adjusted the models also for zone of residence.

TABLE 1. Determinants of elevated (>10 ppt) plasma levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) measured in subjects from the Seveso population from 1993 to 1998[a]

	Plasma TCDD		OR[b]	(95% CI)[b]	*p* value[b]
	<=10 ppt (n = 215)	>10 ppt (n = 78)			
Residence at the date of the accident					
Non-contaminated	73 (34.0%)	4 (5.1)	1.0	-	-
R zone	73 (34.0%)	3 (3.9%)	3.2	(0.5-18.4)	0.20
B zone	36 (16.7%)	27 (34.6%)	25.8	(7.0-95.0)	<0.001
A zone	33 (15.3%)	44 (56.4%)	65.9	(16.6-262.1)	<0.001
Age at the date of the accident					
6 months-8 years	89 (41.4%)	16 (20.5%)	1.0	-	
8-13 years	60 (27.9%)	10 (12.8%)	1.8	(0.6-5.1)	0.27
13-18 years	13 (6.1%)	15 (19.2%)	3.6	(1.1-11.0)	0.03
>18 years	53 (24.7%)	37 (47.4%)	5.9	(2.3-15.1)	<0.001
Gender					
Male	132 (61.4%)	21 (26.9%)	1.0	-	
Female	83 (38.6%)	57 (73.1%)	5.3	(2.5-11.2)	<0.001
Body Mass Index (BMI)[c]					
<21.7 kg/m^2	75 (35.1%)	22 (28.6%)	1.0	-	
21.7-25 kg/m^2	69 (32.2%)	26 (33.8%)	1.6	(0.6-4.0)	0.29
>25 kg/m^2	70 (32.7%)	29 (37.7%)	2.8	(1.0-4.0)	0.04

[a] Lipid adjusted plasma TCDD levels measured approximately 20 years after the Seveso accident

[b] Odds Ratios (ORs), 95% Confidence Intervals (CIs) and *p* values adjusted in multiple regression analysis for gender, and age and zone of residence at the date of the accident

[c] BMI measured at the interview. Total number of subjects varies because of missing values

Chloracne and plasma TCDD levels

Chloracne was nearly four times more frequent in subjects with current plasma TCDD >10 ppt (OR=3.7, 95% CI: 1.6-8.8, adjusted for zone of residence and age at the accident and gender) (Table 2). Among them, 21 chloracne cases and 12 controls had TCDD plasma levels higher than 50 ppt and an OR for chloracne of 20.4 (95% CI: 5.1-81.0), relative to subjects with TCDD<=10 ppt. Subjects with TCDD levels>10 ppt who were 8 years of age or younger at the time of the accident had OR=7.4 (95% CI: 1.8-30.3) for chloracne (Table 3). Among subjects older than 8 years, the OR was 1.3 (95% CI: 0.6-3.1) (p=0.02 for the interaction between age and plasma TCDD).

We evaluated whether subjects' pigmentary characteristics were related to chloracne. Chloracne status exhibited borderline association with eye colour (OR=1.6, 95% CI: 0.9-2.8 for light brown, green, grey or blue vs. dark brown; adjusted for age, gender and zone of residence), while skin colour (OR=0.7, 95% CI: 0.4-1.2 for light vs. medium or dark), hair colour (OR=0.9, 95% CI: 0.5-1.7 for light brown, reddish brown, blonde or red vs. dark brown or black) or skin reaction after one hour in the sun (OR=1.5, 95% CI: 0.7-2.9 for subjects who tended to burn vs. never burn) were not associated with the disease. Interestingly, subjects with TCDD levels>10 ppt, had higher relative

odds of chloracne if they had light (OR=9.2, 95% CI: 2.6-32.5) rather than darker hair colour (OR=2.1, 95% CI: 0.7-6.1, p=0.04 for the interaction between plasma TCDD and hair colour), when compared with those with lower TCDD levels (Table 3).

TABLE 2. Relative odds of chloracne for subjects with elevated (>10 ppt) current plasma levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), measured in subjects from the Seveso population from 1993 to 1998[a]

	Chloracne subjects	**Control subjects**	**OR[b]**	**(95% CI)[b]**
	(n = 98)	(n = 195)		
All subjects				
Plasma TCDD<=10 ppt	70	145	1.0	-
Plasma TCDD >10 ppt	28	50	3.7	(1.6-8.8)
Age at the accident <=8 years				
Plasma TCDD<=10 ppt	38	51	1.0	-
Plasma TCDD >10 ppt	12	4	7.4[c]	(1.8-30.3)
Age at the accident >8 years				
Plasma TCDD<=10 ppt	32	94	1.0	-
Plasma TCDD >10 ppt	16	46	1.3[c]	(0.6-3.1)
Dark hair colour				
Plasma TCDD<=10 ppt	43	65	1.0	-
Plasma TCDD >10 ppt	14	23	2.1[d]	(0.7-6.1)
Light hair colour				
Plasma TCDD<=10 ppt	25	67	1.0	-
Plasma TCDD >10 ppt	13	17	9.2[d]	(2.6-32.5)

[a] Lipid-adjusted plasma TCDD levels measured approximately 20 years after the accident
[b] Odds Ratios (ORs) and 95% Confidence Intervals (CIs) computed using logistic regression models with plasma TCDD, gender, and age and zone of residence at the date of the accident as independent variables
[c] p = 0.02 for the difference between age-specific odds ratios
[d] p = 0.04 for the difference between hair-colour-specific odds ratios

In summary, in the present study we found a strong association between TCDD levels and chloracne occurrence, which persisted after 20 years from the exposure. This was possible because of the long half life of dioxins in humans.[14-16] This study is unique with regard to age and gender distribution of the study subjects, relatively pure exposure to TCDD, availability of epidemiological and clinical data, as well as individual assessment of TCDD plasma levels.

Dioxin levels measured 20 years after the exposure appeared to be positively associated with age at the time of the accident. This is in contrast with what was found in samples collected in Seveso at the time of the accident.[17] In fact, in those samples TCDD levels were highest among the youngest subjects and decreased until approximately 13 years of age, after which there was no change.[17] We found a nearly linear increase of dioxin levels by age, within the age range of our study subjects (Table 1). Differential elimination by age or body dilution of dioxin consequent to body growth in

young children[18] may explain this discrepancy. This finding is important for interpreting the association of dioxin with chloracne. In our study, chloracne occurrence was more strongly associated with plasma TCDD levels among individuals who were younger than 8 years at the accident (OR=7.4, Table 3). This may reflect a higher elimination/dilution of TCDD, as well as an increased sensitivity to chloracnegenic factors in younger subjects. The hypothesis that the endogenous hormonal changes of puberty may favour chloracne development[19], possibly interacting with TCDD-endocrine-like actions,[20] was not supported by our results. In fact, the majority of the chloracne cases were pre-pubescent at the time of the accident, and the highest risk was associated with age younger than 8 years.

We found a strong association between plasma TCDD levels and chloracne among subjects with light hair colour. In addition, subjects with light eye colour exhibited a suggestive, though non-significant, increased risk of chloracne. Individual genetics characteristic are known to modify dioxin toxicity.[21-23] Genetics or environmental factors related to pigmentation may have altered chloracne occurrence in this population. Our finding requires confirmation in larger studies, possibly with standardized measurements of pigmentation characteristics.

In conclusion, 20 years after the Seveso accident, dioxin levels were still elevated in exposed individuals, particularly in females, in subjects who had eaten home-grown animals, and in individuals with older age, higher BMI, and residence near the accident site. Plasma dioxin was strongly associated with chloracne. This association was modified by age and pigmentation characteristics, possibly reflecting an increased sensitivity to chloracnegenic factors or differential dioxin clearance.

References

1. Bertazzi P.A., Bernucci I., Brambilla G., Consonni D. and Pesatori A.C. (1998) Environ Health Perspect. 106 Suppl 2, 625.
2. Bertazzi P.A., Consonni D., Bachetti S., Rubagotti M., Baccarelli A., Zocchetti C. and Pesatori A.C. (2001) Am.J Epidemiol. 153, 1031.
3. Baccarelli A., Mocarelli P., Patterson D.G., Jr., Bonzini M., Pesatori A.C., Caporaso N. and Landi M.T. (2002) Environ.Health Perspect. 110, 1169.
4. Pesatori A.C., Consonni D., Bachetti S., Zocchetti C., Bonzini M., Baccarelli A. and Bertazzi P.A. (2003) Ind.Health 41, 127.
5. Baccarelli A., Pesatori A.C., Consonni D., Mocarelli P., Patterson D., Caporaso N.E., Bertazzi P.A. and Landi M.T. (in press) British Journal of Dermatology.
6. Seveso Chloracne Panel (1977), Milan, 11-12 July.
7. Patterson D.G., Jr., Hampton L., Lapeza C.R., Jr., Belser W.T., Green V., Alexander L. and Needham L.L. (1987) Anal.Chem. 59, 2000.
8. Hornung R.W. and Reed L.D. (1990) Appl Occup Environ Hyg 5, 48.
9. Phillips D.L. (1989) Arch.Environ.Contam Toxicol. 18, 508.
10. Steenland K., Calvert G., Ketchum N. and Michalek J. (2001) Occup Environ Med 58, 641.
11. Neubert R., Maskow L., Triebig G., Broding H.C., Jacob-Muller U., Helge H. and Neubert D. (2000) Life Sci. 66, 2123.
12. Michalek J.E., Rahe A.J., Kulkarni P.M. and Tripathi R.C. (1998) J Expo Anal Environ Epidemiol 8, 59.

13. Landi M.T., Needham L.L., Lucier G., Mocarelli P., Bertazzi P.A. and Caporaso N. (1997) Lancet 349, 1811.
14. Geusau A., Tschachler E., Meixner M., Papke O., Stingl G. and McLachlan M. (2001) Br J Dermatol 145, 938.
15. Pirkle J.L., Wolfe W.H., Patterson D.G., Needham L.L., Michalek J.E., Miner J.C., Peterson M.R. and Phillips D.L. (1989) J Toxicol.Environ.Health 27, 165.
16. Michalek J.E., Pirkle J.L., Needham L.L., Patterson D.G., Jr., Caudill S.P., Tripathi R.C. and Mocarelli P. (2002) J.Expo.Anal.Environ.Epidemiol. 12, 44.
17. Eskenazi B., Mocarelli P., Warner M., Needham L., Patterson D.G., Jr., Samuels S., Turner W., Gerthoux P.M. and Brambilla P. (2004) Environ Health Perspect 112, 22.
18. Kreuzer P.E., Csanady G.A., Baur C., Kessler W., Papke O., Greim H. and Filser J.G. (1997) Arch.Toxicol. 71, 383.
19. Silbergeld E.K. (1996) Chemicals and chloracne, in Dermatotoxicology, Marzulli, F. N., and Maibach, H. I., Eds., Taylor & Francis, Washington, DC, 249.
20. Baccarelli A., Pesatori A.C. and Bertazzi P.A. (2000) J.Endocrinol.Invest 23, 771.
21. Landi M.T., Bertazzi P.A., Baccarelli A., Consonni D., Masten S., Lucier G., Mocarelli P., Needham L., Caporaso N. and Grassman J. (2003) Carcinogenesis 24, 673.
22. Landi M.T. and Baccarelli A. (2003) Cancer Epidemiol.Biomarkers Prev. 12, 1116.
23. Baccarelli A., Pesatori A.C., Masten S.A., Patterson D.G., Jr., Needham L.L., Mocarelli P., Caporaso N.E., Consonni D., Grassman J.A., Bertazzi P.A. and Landi M.T. (2004) Toxicol Lett 149, 287.