

Exposure to polychlorinated biphenyls and hexachlorobenzene, semen quality and testicular cancer risk

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Abstract

Purpose We carried out a case–control study to investigate the possible role of occupational and environmental exposure to endocrine disruptors in the onset of testicular cancer (TC).

Methods We evaluated 125 TC patients and 103 controls. Seminal fluid examination and organochlorine analysis were performed in all subjects. Cases and controls were also interviewed using a structured questionnaire to collect demographic information, residence, andrological medical history and dietary information.

Results We found that a higher level of reproductive tract birth defects was associated with a higher risk of TC. With regard to diet, cases reported a higher consumption of milk and dairy products than controls. Overall, there was a statistically significant increase in TC risk in cases with detectable values of total polychlorinated organic compounds against controls (14.4 vs. 1.0 %; $p < 0.001$). TC patients with detectable levels of organochlorines had lower mean semen parameters than those

with undetectable levels, although this difference was not statistically significant.

Conclusion The International Agency for Research on Cancer recently included dioxin-like polychlorinated biphenyls (PCBs) in Group 1 of known human carcinogens. Our study confirmed and identified various risk factors for testicular cancer: cryptorchidism, consumption of milk and dairy products, parents' occupation and serum concentration of hexachlorobenzene and PCBs and, for the first time, we showed the correlation between semen quality and the serum concentration of these pollutants.

Keywords Polychlorinated biphenyls · Hexachlorobenzene · Testicular cancer · Semen quality · Environmental exposure

Introduction

The international incidence of testicular cancer (TC) varies considerably among different countries and ethnic groups. Like many other western countries, Italy has experienced an increased incidence in recent years: the standardised incidence rate rose from 3.7 cases per year per 100,000 inhabitants in 1993–1995 to 5.2 in 2003–2005, a 40.5 % increase [1]. Recent projections estimated that by 2025 there would be 23,000 new cases of testicular cancer every year in Europe, a rise of 24 % from 2005. The most rapid rise is predicted for southern Europe, particularly Italy [2].

The reasons for this increase are unknown, as the risk factors for this disease are poorly understood. Various predisposing factors have been identified such as cryptorchidism, contralateral testicular cancer and a family

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history of testicular cancer. However, epidemiological studies since 1980 have suggested that other factors, such as in utero exposure to high oestrogen levels, might influence the onset of testicular cancer. Various case–control studies on testicular cancer patients have evaluated the effects of hormone therapy during pregnancy, albeit with contrasting results [3]. Prenatal exposure to environmental pollutants has also been suggested to be of etiological significance. The role of sex hormones in testicular carcinogenesis has thus aroused considerable interest, with recent studies investigating exposure to endocrine disruptors (EDs): chemicals found in the environment with an oestrogen-like or anti-androgenic endocrine action such as organochlorines (OCs). The latter include polychlorinated biphenyls (PCBs), a group of persistent synthetic lipophilic compounds previously used in consumer and industrial products that can still be detected in the air, water, marine beds, fish and wild animals. This group comprises 209 aromatic congeners (some of which are dioxin like), all containing a biphenyl ring with from 1 to 10 chlorine atoms. The half-life of these compounds depends on the degree of chlorination and can vary from 7 to 30 years. The International Agency for Research on Cancer (IARC) recently included dioxin-like PCBs in Group 1 of known human carcinogens, based in part on strong evidence of the mechanism of carcinogenesis mediated by the aryl hydrocarbon receptor (AhR), which is identical to that of 2,3,7,8-tetrachlorodibenzoparadioxin, and evidence of carcinogenicity in laboratory animals [4]. These substances are mainly ingested through contaminated food. In fact, various foods, especially milk and cheese, can contain detectable quantities of organochlorines as well as of endogenous hormones such as oestrogen and progesterone.

Several studies have investigated the association between the presence of organochlorines in serum and the risk of testicular cancer [5–11]. Literature evidence does, however, demonstrate that reproduction and pre- and post-natal development are particularly sensitive to the endocrine effects of EDs, although the biological mechanisms underlying these correlations, any coexisting genetic susceptibility and risk factors and any diseases that might be associated with exposure to EDs are still to be clarified.

On the basis of recent epidemiological evidence (from IARC), we carried out a case–control study to investigate the possible role of environmental exposure to PCBs and HCB in the onset of testicular cancer. We analysed the serum concentration of these compounds in testicular cancer patients and controls, alongside occupational risk factors, using a job exposure matrix (JEM). We also studied, for the first time, semen quality in correlation with the serum concentration of these pollutants.

Materials and methods

Study population

This case–control study of Caucasian males was approved by the University Hospital “Umberto I” Institutional Review Board. Written informed consent was obtained from all study participants. We recruited 125 testicular cancer patients aged 29.6 ± 5.9 years (seminoma and non-seminoma) attending the Laboratory of Semiology-Sperm Bank at the Department of Experimental Medicine, University of Rome ‘La Sapienza’, for semen cryobanking. All patients were studied ~1 month after orchiectomy and before beginning chemo- or radiotherapy. The control group consisted of 103 subjects aged 31.3 ± 6.6 years recruited from healthy men undergoing an andrological examination and semen analysis in the same department for check-ups under a nationwide preventive screening campaign. Cases and controls were well balanced for BMI and place of residence. Seminal fluid examination and organochlorine analysis were performed in all subjects.

Cases and controls were also interviewed face-to-face by specifically trained medical doctors using a structured questionnaire to collect demographic information, residence prior to diagnosis and andrological medical history (presence of congenital defects such as cryptorchidism and others, history of trauma and previous infections). The questionnaire also included a detailed reconstruction of occupational history, lifestyle and other environmental factors involving activities with suspected exposure to OCs. Detailed dietary information was collected, including consumption of potentially contaminated food items: milk and dairy products, fish, fruit, vegetables and meat. The men were asked about the frequency of consumption (every day, at least once per week, at least once per month, less than once per month, never) for each food item. The five consumption frequencies were then collapsed into two classes (rare or frequent) for each food item as described elsewhere [12]. For example, in Italy the consumption of dairy products once per week is considered rare, while the consumption of fruit and vegetables every day is classified as frequent [12].

Occupational exposure investigated all jobs that subjects had held for at least 1 year before diagnosis. Information on occupation included work sector, specific job and chemical exposure reported by the subject. The interview also investigated parental occupational exposure to EDs in the periconceptional period. Occupational exposure to groups of EDs was evaluated by a specific job exposure matrix (JEM) developed by Van Tongeren et al. [13] for the purpose of classifying jobs in relation to exposure to given groups of EDs (e.g. pesticides, phthalates). For each occupation reported by the subject, the corresponding job

in the matrix was found, and the subject was assigned an exposure probability level, categorised as 0 (no exposure) or ≥ 1 (medium or high exposure). We considered all subjects reporting occupations with a JEM score of 1 or 2 as “probably exposed”. As a job may involve exposure to more than one group of EDs, we used the number of ED groups involved in each job (0, 1, >1) as a summary measure of ED exposure.

The questionnaire also included information on the subjects’ perinatal characteristics (reproductive birth defects, parity, mother’s age at birth and breastfeeding). Prenatal residential information for the subjects’ parents was also collected. Subjects were asked to classify parental occupational exposure to EDs on the basis of the JEM.

Semen analysis

Semen samples were collected by masturbation after 3–5 days of abstinence. All samples were allowed to liquefy at 37 °C for 60 min and were then assessed according to 2010 World Health Organisation guidelines [14]. The following variables were taken into consideration: ejaculate volume (ml), sperm concentration ($N \times 10^6/\text{mL}$), total sperm number ($N \times 10^6/\text{ejaculate}$), total motility (%) and morphology (% abnormal forms). In cases of azoospermia (no sperm in the ejaculate), the analysis was performed twice and the diagnosis was made only after having carefully checked the entire post-centrifuge pellet.

Organochlorine analysis

Serum samples were analysed at the Laboratory for Environmental and Toxicological Testing, Salvatore Maugeri Foundation, Pavia. The persistent organochlorine pollutants investigated in the study included the PCB congeners (31, 28, 52, 77, 153, 126, 180, 169, 170) and hexachlorobenzene. Organochlorines were evaluated following the procedure we described in a previous paper [12]. A fast, reliable analytical method was previously developed and validated [15]. Briefly, 2 mL of methanol was added to the same volume of serum and the solution was vortexed for 30 s. The extraction was performed using 5 mL ethyl ether/hexane (1:1, v/v). The organic phase was separated and the extraction procedure was repeated twice. Organic phases were evaporated under a stream of nitrogen to a volume of about 500 μL . This residue was then purified with Bond Elut PCB after prior treatment of the cartridge with 1 mL hexane. The extract was eluted with 3 mL hexane and then with 3 mL hexane:ethyl ether (1:1, v/v). The eluates were collected and dried in a stream of nitrogen. The dry residue was re-dissolved in 100 μL hexane and analysed using gas chromatography–mass spectrometry (GC–MS) with a Shimadzu (Shimadzu Deutschland GmbH, Duisburg,

Germany) GCMSQP-QP5050A gas chromatograph mass spectrometer equipped with an autoinjector/auto-sampler AOC-20.

Statistical analyses

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS ® v. 17.0 for Windows) and STATA-9. The serum concentrations of the organochlorine pollutants were treated as dichotomous variables.

As the serum OC concentrations were often below the level of detection (LOD), polychlorinated biphenyls and HCB values were grouped as being below or above this cutoff point (LOD = 0.2 ng/ml). Continuous and categorical variables were created for the independent variables age, residence (urban/rural) and education. Cross-tabulations were run for the likelihood ratio Chi-squared test and Fisher’s exact test, when appropriate. Additional variables from the crude analyses and TC covariates were added to the model and the estimated coefficients were compared with and without the additional variable to see if there were any differences. The associations between the different exposure variables and the risk of testicular cancer were estimated by logistic regression and expressed as odds ratio (OR) and 95 % confidence interval (CI). Interview data were used to evaluate the role of the different kinds of reported exposures (occupational, dietary, etc.) in relation to the risk of testicular cancer. First, we calculated the OR and 95 % (CI) for the characteristics of the infant cases and controls and their parents. Data were also adjusted (OR_{adjusted}) for possible confounding factors such as age and educational level. ANOVA was used to test the difference in mean semen parameters between cases and controls.

In contingency tables, for ordered categorical variables, Chi-squared test was used to assess differences in the prevalence of oligozoospermic TC patients among detected and undetected PCB groups, with significance set at 0.05.

We carried out the Chi-squared test to see if there were any differences between the cases and controls in smokers, non-smokers and ex-smokers.

Results

The perinatal and congenital characteristics of cases and controls and their differences based on Chi-squared analysis are reported in Table 1. In comparing all TC cases to controls, birth weight, mother’s parity, defined as the number of the mother’s births prior to subject, and history of perinatal breast feeding were similar ($p \geq 0.05$), while cryptorchidism was significantly more common among the cases ($p = 0.019$). This result is consistent with literature

Table 1 Perinatal and congenital characteristics of TC cases and controls

	TC cases 125 pts N (%)	Controls 103 pts N (%)	<i>p</i> value
Perinatal characteristics			
Birth weight (Kg) <2.5 kg	5 (4.5)	2 (2.1)	0.38 ^a
Breastfed	102 (84.3)	89 (87.3)	0.32 ^a
Mother's parity* ≤1 son	15 (12.0)	8 (9.3)	0.29 ^a
Congenital birth defects			
Cryptorchidism	15 (12.0)	3 (2.9)	0.019 ^b
Hypospadias	5 (4.0)	2 (1.9)	0.38 ^a

* Number of mother's births prior to subject

^a Not significant^b *p* < 0.05

evidence, as reproductive tract birth defects are an established risk factor for TC.

With regard to semen parameters, mean values for total sperm number and total motility were significantly lower in cases (*n* = 122) than in controls (*n* = 101). The percentage of abnormal forms was higher in patients than in controls (Table 2). We found three azoospermic men among the TC patients and two in the control group. Azoospermic patients were excluded from the statistical analysis and evaluated separately.

Table 3 shows the risk of TC in relation to possible occupational exposure to EDs evaluated by JEM in participants and their parents and according to dietary habits. Six case fathers and one control father had been exposed to EDs (OR 5.49; 95 % CI 0.65–46.45). There was a non-statistically significant increase in the risk of TC associated with phthalate exposure of participants (OR 1.34; 95 % CI 0.42–4.22) and their fathers (OR 1.50; 95 % CI 0.52–4.30). There was also an increased risk, although again not statistically significant, associated with mothers' exposure to pesticides (OR 2.08; 95 % CI 0.39–10.95). There was a significant increase in the OR for a number of probable occupational exposures to EDs. In fact, six of the cases but none of the controls had probably been exposed to EDs (Fisher's exact test *p* = 0.007).

With regard to diet, cases reported a higher consumption of milk and dairy products (OR 2.33; 95 % CI 1.02–5.29) than control subjects. This association was statistically significant. There was also an association between meat consumption and TC (OR 2.21; 95 % CI 0.93–5.26), although this was not statistically significant. No association was found between fish consumption and TC. The frequent consumption of fruit and vegetables did not increase the risk of TC, and in fact seemed to be inversely correlated (OR 0.53; 95 % CI 0.31–0.93). Furthermore, in our study we found a balanced ratio of smokers, non-smokers and ex-smokers between cases and controls, with no significant differences (*p* = 0.653). The serum concentration of each OC was evaluated using Fisher's exact test, as the number of samples in which they could be detected was too small. A detected concentration of the sum of the 9 analysed PCB congeners was found in 16 TC patients and no controls. This difference was statistically significant (*p* < 0.001). HCB was detected in five cases and one control; this difference was not statistically significant (OR 4.25; 95 % CI 0.49–36.97; *p* = 0.22). Overall, there was a statistically significant increase in TC risk in cases with detectable values of total polychlorinated organic compounds (14.4 %) with respect to controls (1.0 %) (*p* < 0.001).

To analyse the relationship between serum concentration of PCBs/HCB and semen quality in the TC patients, we divided them into two subgroups of detected (*n* = 17) and undetected (*n* = 105) serum values of polychlorinated organic compounds. TC patients with detectable values of PCBs/HCB showed lower mean values for sperm concentration, total sperm number and total motility and a higher percentage of abnormal forms than those with undetectable levels, although this difference was not statistically significant (Table 4). The three azoospermic patients were evaluated separately; only one of these had detectable levels of PCB.

Specifically, the percentage of TC patients with impaired spermatogenesis (<39 × 10⁶ total sperm number) according to WHO 2010 was 50.0 % in those with detectable values of PCBs and 25.7 % in those with undetectable levels (Fig. 1). Furthermore, detectable serum levels of each of the examined PCB congeners were found in a higher

Table 2 Age and sperm parameters (mean ± SD) in TC cases and controls excluding azoospermic subjects

	N (Years)	Volume (ml)	Sperm concentration (× 10 ⁶ /ml)	Total sperm number (× 10 ⁶ /ejaculate)	Total motility (%)	Abnormal forms (%)
TC cases	122 (29.62 ± 5.94)	3.36 ± 1.66	38.78 ± 36.48	119.27 ± 119.84	36.55 ± 16.38	77.15 ± 10.44
Controls	101 (31.31 ± 6.60) ^b	3.45 ± 1.60 ^a	75.54 ± 57.35 ^c	243.20 ± 194.53 ^c	46.66 ± 13.03 ^c	72.66 ± 7.98 ^b

^a Not significant^b *p* < 0.05^c *p* < 0.01

Table 3 Occupational and dietary exposure to EDs in participants and their parents evaluated by JEM and food-consumption questionnaire

	TC cases		Controls		OR	CI	OR* _{adjusted}	CI
	(n = 125)	(%)	(n = 103)	(%)				
Probable paternal occupational exposure to EDs								
Pesticides	13	11.5	13	13.1	0.86	(0.37–1.95)	0.78	(0.32–1.89)
Polychlorinated organic compounds	6	5.3	1	1.0	5.49	(0.65–46.45)	3.78	(0.43–32.86)
Phthalates	10	8.8	6	6.0	1.50	(0.52–4.30)	1.10	(0.36–3.38)
Alkylphenolic compounds	9	7.9	8	8.0	0.98	(0.36–2.65)	0.79	(0.27–2.33)
Missing	12		3					
Probable maternal occupational exposure to EDs								
Pesticides	5	4.0	2	1.9	2.08	(0.39–10.95)	1.97	(0.36–10.66)
Polychlorinated organic compounds	1	0.8	1	1.0	0.81	(0.05–13.16)	1.00	(0.57–17.57)
Phthalates	12	9.5	15	15.0	0.59	(0.26–1.34)	1.03	(0.26–4.11)
Alkylphenolic compounds	5	4.0	4	3.9	1.01	(0.26–3.89)	1.54	(0.49–4.80)
Missing	1		2					
Probable occupational exposure to EDs								
Pesticides	4	3.2	6	5.8	0.53	(0.14–1.94)	0.50	(0.13–1.93)
Polychlorinated organic compounds	6	4.8	0	0.0	<i>p</i> _{fisher} = 0.007		<i>p</i> _{fisher} = 0.02	
Phthalates	8	6.4	5	4.8	1.34	(0.42–4.22)	0.92	(0.28–3.01)
Alkylphenolic compounds	5	4.0	7	6.7	0.57	(0.17–1.85)	0.46	(0.13–1.54)
High-frequency consumption of possible ED contaminated foods								
Milk and dairy products	23	18.4	9	8.8	2.33	(1.02–5.29)	2.37	(1.01–5.52)
Meat	20	16.0	8	7.9	2.21	(0.93–5.26)	2.17	(0.85–5.53)
Fruit and vegetables	69	55.2	71	69.6	0.53	(0.31–0.93)	0.56	(0.31–1.00)
Fish	89	71.2	68	66.7	1.23	(0.70–2.17)	1.43	(0.79–2.58)

* OR adjusted for age and educational level of participants

Table 4 Semen parameters (mean ± SD) in TC cases with or without detectable levels of PCBs/HCB

	Detected	Undetected	<i>p</i> value
No patients	17	105	
Volume (ml)	3.33 ± 1.2	3.36 ± 1.7	0.94 ^a
Sperm concentration (×10 ⁶ /ml)	28.73 ± 33.4	40.19 ± 36.8	0.25 ^a
Total sperm number (×10 ⁶ /ejaculate)	108.59 ± 172.8	123.68 ± 109.2	0.63 ^a
Total motility (%)	32.35 ± 16.0	37.23 ± 16.4	0.25 ^a
Abnormal forms (%)	78.94 ± 10.8	76.86 ± 10.4	0.45 ^a

^a Not significant

percentage of TC cases with impaired spermatogenesis than in normozoospermic patients. The congener PCB 153 was found more.

Discussion

Testicular and germ cell development is a highly complex process which is regulated from the initial embryonic

stages. These phases are particularly sensitive to endogenous and exogenous hormones, such as endocrine disrupters, which could disrupt the homeostasis necessary for the normal development and differentiation of these cells by acting on post-transcriptional regulators [16] or even by inducing epigenetic changes [17]. EDs are a diverse group of compounds which all have the potential to interfere with the endocrine system, acting through different mechanisms. EDs include organochlorine pesticides and PCBs, both of which are ubiquitous, persistent lipophilic pollutants.

Various studies have evaluated if environmental pollutants and other biological factors might interfere with hormone balance during the prenatal and post-natal period, thus leading to diseases such as testicular cancer in adults. The few studies of the association between testicular cancer and serum concentration of organochlorines conducted to date mainly concern northern European and US populations. For this reason, we conducted a case–control study in Italy, analysing the concentration of PCBs and HCB in the serum of TC cases and of controls using the job exposure matrix (JEM) to evaluate occupational risk factors.

A number of studies have examined the possible association between serum concentration of PCBs and the

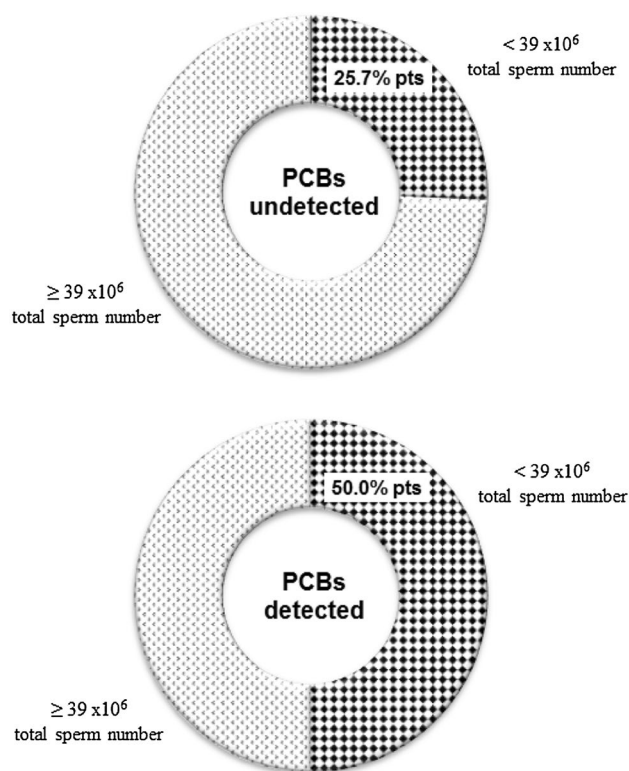


Fig. 1 Percentage of TC cases with detected and undetected serum values of PCBs with total sperm number <39 and $\geq 39 \times 10^6$ per ejaculate

development of testicular cancer. A Swedish study [6] analysed the concentration of 37 PCB congeners in 58 testicular cancer cases and 61 controls and in the serum of 44 case mothers and 45 control mothers. No significant differences were found between cases and controls, but the concentration of PCBs was higher in case mothers. McGlynn et al. [10] evaluated the relationship between PCB and testicular cancer in 736 cases and 918 controls, finding an inverse relationship between testicular cancer and 8 of the 11 PCBs analysed. This study therefore did not support the theory that exposure to PCBs increases the risk of testicular cancer. Another case–control study conducted by Purdue et al. [11] in the same year analysed the pre-diagnosis serum concentration of 11 organochlorines and 34 PCB congeners in 49 cases and 51 controls. This study therefore did not find a clear association between PCB levels and testicular cancer, perhaps due to the small caseload.

Serum concentrations of the PCBs analysed in our study were higher in the cases than in the controls, and the increase in the risk of TC was statistically significant. In contrast, we found a non-statistically significant increase in the risk of TC for participants with higher serum concentrations of HCB. This fungicide has previously been assessed in relation to TC in three studies, but none found

any evidence of an association [5, 9, 11]. On the whole, we found statistically significant differences between cases and controls as regards total PCBs and total polychlorinated organic compounds (HCB + PCBs) ($p < 0.001$). This result should be further investigated, in light of the recent upgraded classification of PCBs as human carcinogens [4].

We found no association between TC and maternal factors (e.g. parity, breastfeeding) or perinatal factors (e.g. weight at birth). Literature evidence as to the possible role of these factors is still contradictory [18]. We found a higher risk of TC in patients with reproductive diseases, especially those with cryptorchidism, confirming literature reports [19].

We also found an association between TC and consumption of milk and dairy products. This is in accordance with literature studies that have identified dairy products and milk consumption as an important risk factor for testicular cancer [20–22]. In fact, the consumption of milk and dairy products is highest in Scandinavian countries, which have the highest incidence of testicular cancer; in contrast, Asian and African countries have the lowest consumption of these products and the lowest incidence of testicular cancer. It could therefore be postulated that milk and dairy products containing high levels of oestrogens and progesterone might be associated with the development of testicular cancer [22]. In fact, most studies have found that meat, fish and dairy products are the most important contributors to the total dietary intake of organochlorines [23, 24]. The main mechanism by which OCs enter the food chain seems to be contamination from pesticides sprayed on plants that are subsequently ingested by animals. These substances build up in the fatty tissues, so consumption of contaminated food and soil by herbivores can lead to their accumulation in the fat of their meat and their milk. Milk and dairy products are one of the major sources for the bioaccumulation of lipophilic EDs such as PCBs. Other foods of animal origin, such as meat, showed a non-statistically significant increased risk for TC. We did not find any association between the consumption of fish and TC. This might suggest that contaminants tend to be less bioaccumulated in this kind of food in the Mediterranean area than in northern European waters. The bioaccumulation of contaminants in fish, especially lake fish, is well documented in various Nordic countries, but environmental data are scanty for southern Europe and Italy.

We did not find any association between TC and consumption of fruit and vegetables. Fruit and vegetable consumption is regarded as protecting against cancer in general, and hormone-related cancers in particular, by reducing the enterohepatic recirculation of oestrogens [25]. However, some studies have shown a possible risk associated with EDs such as pesticide residues and phytoestrogens that might be present in vegetables, with oestrogenic and

anti-androgenic effects which could play a role in testicular dysgenesis syndrome [26].

We found no significant association between TC and occupational exposure to OCs. Given the early action of risk factors in the development of the testes, occupational exposure is probably not related to the onset of the disease, especially in younger men. We found some association with parents' occupation. This was particularly evident among fathers who had probably been exposed to organochlorine compounds. There was also an association with case mothers exposed to pesticides in comparison with control mothers, although this was not statistically significant. The possible role of parental exposure has been examined in a small number of studies [27]. Paternal and maternal exposure in prenatal life probably affects the developing testis, but to date, the evidence is unclear and the issue should be further investigated. The associations observed in our study should be considered only tentative and do not constitute evidence of a cause–effect relationship between the exposure variables identified as associated with TC, except for factors which might have a strong association with TC (e.g. cryptorchidism). One limitation is that the serum concentrations of PCBs and HCB were lower than those found in Nordic populations. This may be partly due to lower exposure to PCBs through dietary sources in Italy in comparison with Inuit and Scandinavian populations [5, 12].

This research paper also has some advantages. To our knowledge, no studies have previously investigated the semen quality of TC patients in relation to serum concentration of PCBs/HCB. We found that although our TC cases had mean semen values within normal limits, sperm concentration and motility were lower and abnormal forms more frequent in comparison with the controls. TC patients with detectable OC levels had lower mean semen parameter values compared to those with undetectable levels, although this difference was not statistically significant. When considering PCBs only, oligozoospermia in patients with detectable levels was double that of patients with undetectable values; this difference was statistically significant.

Spermatogenesis, which is regulated by the endocrine system, could be particularly sensitive to organochlorines that mimic male hormones and interfere with the normal function of the hypothalamic–pituitary–gonadal axis. Various recent studies have considered the potential effect of pesticides on spermatogenesis, given the literature evidence of a longitudinal decline in semen quality [28] in various geographic areas, including Italy [29], New Zealand [30], Tunisia [31], France [32], India [33] and Israel [34]. This reduction may be due to genetic differences, environmental factors, or both [35]. Some studies found genetic polymorphisms which might influence semen quality after exposure to environmental pesticides, suggesting that changes

in genes related to pesticide metabolism could affect susceptibility to sperm abnormalities as a result of exposure [9, 36]. The results of our study are particularly interesting in light of these findings, as they could suggest the existence of subjects who are genetically predisposed and thus more sensitive to endocrine disruptors, with a higher risk of impaired semen quality and higher incidence of testicular cancer.

In our TC patients, we found a higher percentage with a total sperm number $<39 \times 10^6$ in those with detectable PCB levels than in those with undetectable values. These interesting results indicate the need for greater knowledge of the relationship between semen quality, environmental factors and the onset of testicular cancer.

In conclusion, our study confirmed and identified various risk factors for testicular cancer: reproductive disorders, especially cryptorchidism; consumption of milk and dairy products, which are one of the major sources of bio-accumulating lipophilic EDs such as PCBs; parents' occupation and serum concentration of HCB and PCBs.

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Conflict of interest The authors have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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