Polychlorinated biphenyl serum levels in subjects with hepatocellular carcinoma as compared with the general population

Claudia Zani[1], Nazario Portolani[2], Umberto Gelatti[1], Roberto Bergonzi[3], Pietro Apostoli[3],Francesco Donato[1], for the Brescia HCC and PCB Study Group[4]

ABSTRACT

BACKGROUND: Polychlorinated biphenyls (PCBs) have been recognised as human carcinogens and cause liver cancer in animal experimental studies. However, their association with hepatocellular carcinoma (HCC) has not yet been investigated. This study aimed to evaluate the serum PCB concentration in HCC patients and in healthy subjects of the general population living in Brescia, North Italy, a highly industrialized area with heavy PCB environmental pollution due to the presence of a PCB producing factory.

METHODS: Lipid-adjusted PCB concentrations, computed as the sum of 24 congeners, were measured in the serum of 101 HCC patients and in 101 healthy subjects of the same age and gender.

RESULTS: Hepatitis B and C virus infection and history of heavy alcohol intake were found, alone and combined, in 87% of HCC patients. No difference was found in PCB serum concentration of HCC patients with and without, and according to, the major risk factors for liver disease. No significant difference was observed in serum total PCB concentration between HCC patients (median: 1081; range: 287.0-3182.0 ng/g lipid) and healthy subjects (median: 1199.3; range: 225.7-22825 ng/g lipid). PCB congeners 118, 138, 153, 156, 180 and 194 were the only ones found over the detection limit in at least 30% of HCC patients. The serum level of PCB 118, but not that of other congeners, was higher in HCC patients than in healthy subjects.

CONCLUSIONS: These findings do not support the hypothesis that PCBs play an important role in HCC development, although a contribution by some specific congeners cannot be ruled out.

Key words: Polychlorinated biphenyls, hepatocellular carcinoma, risk factors, environmental pollution, persistent organic pollutants.

(1) Institute of Hygiene, Epidemiology and Public Health, Department of Medical and Surgical Specialities, Radiological Science and Public Health, Brescia University, Brescia, Italy

(2) Department of Medical and Surgical Sciences, Brescia University, Brescia, Italy

(3) Institute of Occupational Health and Industrial Hygiene, Brescia University, Brescia, Italy

(4) Participants in the Brescia HCC and PCB Study Group: Brescia University: G.L Baiocchi and G.A.M. Tiberio, Spedali Civili di Brescia: L. Bettini, G. Pelizzari; Fondazione Poliambulanza di Brescia: A. Salmi and G. Lanzani

CORRESPONDING AUTHOR: *Claudia Zani, Università di Brescia, Dipartimento di Specialità medico chirurgiche, Scienze radiologiche e Sanità Pubblica, Università degli Studi di Brescia 11 Viale Europa, 25123 Brescia, Italy. Tel: +39 030 3717690. Fax: +39 030 3717688.*

E-mail: claudia.zani@med.unibs.it

DOI: ***10.2427/10020***

Accepted on 6 August, 2014; Published as Online First 10 December, 2014

FINANCIAL SUPPORT: *This study was funded by the "Osservatorio per lo studio, la prevenzione e la cura delle patologie epatiche di interesse chirurgico" of the University of Brescia" (Head: Prof. N. Portolani).*

INTRODUCTION

Polychlorinated biphenyls (PCBs) are 209 possible congeners, produced from the 1930s to the 1980s, which persist in the environment and accumulate in plants and animals and are included among persistent organic pollutants (POPs). They have been classified by the International Agency for Research on Cancer (IARC) as carcinogens for humans, with sufficient evidence for melanoma and limited evidence for non-Hodgkin's lymphoma and breast cancer [1].

PCBs are a group of compounds with different chemical and toxicological properties. The 12 congeners with a strong affinity for the Aryl hydroxylase receptor (AhR) have a toxicological profile similar to 2,3,7,8 polychlorinated dibenzo-p-dioxin (TCDD), though with lower toxic equivalency factors (TEFs), and therefore they are classified as dioxin-like (DL)-PCBs [2]. In-vitro and in-vivo assays have shown that PCBs can induce gene mutations, chromosome breaks, oxidative stress and genotoxic effects [3]. Some PCB mixtures and single congeners, especially PCB 118, increased the incidence of liver and other cancers in rats, mainly due to their dioxin-like effects, with a dose-response effect [4,5].

A chemical factory produced PCBs in Brescia, a highly industrialized town in Northern Italy with about 200,000 inhabitants, from 1930 to 1984. These compounds polluted the soil of nearby agricultural areas via irrigation channels, entered the food chain and thus accumulated in human beings, some of whom showed up to 15-fold higher PCB serum levels than the reference values for industrial areas in Italy [6,7]. Moreover, the province of Brescia shows one of the highest incidence rates of liver cancer, particularly hepatocellular carcinoma (HCC), in Italy [8]. A large case-control study carried out in 1995-2001 showed that hepatitis C virus (HCV), hepatitis B virus (HBV) and consumption of more than 60 grams of ethanol per day for at least one decade were responsible for about 90% of the total cases occurring in the area [9], in agreement with findings from other South European countries [10, 11]. A role of environmental toxins, alone or as co-factors, in HCC development cannot be ruled out, however, as shown by the recently observed association between liver cancer and the pesticide DDT [12]. As far as we know, no studies on the association between PCB exposure and HCC in humans have as yet been published.

The aim of this research was to investigate the serum concentration of PCB congeners in HCC patients according to the major risk factors for the disease and, for comparison, in a sample of healthy subjects living in the same area.

MATERIALS AND METHODS

A detailed description of the study design and some data regarding HCC cases are reported in Zani et al.[13]. Briefly, we recruited consecutively, from April 2007 to March 2008, in the two main hospitals in the area 101 patients, residing in the Brescia area and aged up to 80 years, with a first diagnosis of HCC, before they underwent any treatment for the disease (incident cases).The patients were interviewed at the hospital by a trained physician on demographic variables, weight and height, past weight lost, residential and occupational history, and history of alcohol intake. Clinical data were also available. This case series included the 59 HCC patients in whom we investigated the correlation between PCB concentrations in serum, liver and subcutaneous fat [13].

To allow a comparison, we selected 101 healthy subjects, who were part of a random sample of 579 individuals included in a survey carried out in 2003, to determine the serum concentration of PCBs in the adult general population living in the area [6,7]. The healthy subjects were chosen at random from the list of the total subjects included in the survey, after having excluded those occupationally exposed to PCBs, and were matched with HCC cases by age (+5 years) and gender in a 1:1 ratio. Participants were face-to-face interviewed by trained nurses on demographic variables, weight and height, residential history and alcohol intake.

The Ethics Committees of the main hospital in the area and the Local Health Unit approved the project, and each participant provided informed consent.

A 20 ml blood sample was collected under fasting conditions from both HCC patients and healthy subjects, and the serum was separated by centrifugation and stored at –80°C until determination of PCB congeners.

The PCB congeners investigated were

the most commonly searched for in humans exposed to occupational or environmental organochlorinated compounds according to the WHO classification[14]: 28, 31, 52, 77, 81, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 189, 194, 206 and 209. PCBs were measured using a Hewlett-Packard 6890N gas chromatograph coupled with an MSD HP 5973 (electron impact ionization, mass filter: quadrupole). Total PCB serum concentration was calculated as the sum of the 24 PCB congeners measured in serum. For the 12 dioxin-like congeners (77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189), the toxic equivalent (TEQ) was computed using the toxic equivalency factors (TEFs) according to Van den Berg et al. [14]. The limit of quantification in serum varied between PCBs but was generally less than 0.1 ng/ml for each congener. In subjects with values under the limit of quantification, we attributed a concentration of half the quantification limit (0.05 ng/ml) for each PCB congener if it was found in at least 30% of the subjects enrolled, otherwise we attributed a null value. Since PCB serum concentration is influenced by the amount of lipids in the medium, the ratio of PCB concentration to the total lipid levels was computed (lipid-adjusted PCB concentration) and expressed as ng/g lipid. We calculated the total lipid concentration in the serum from cholesterol and triglyceride levels using the formula suggested by Phillips et al. [15].

We also investigated the main risk factors for HCC in the subjects. The presence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection was evaluated by testing the sera for HBsAg (antigen for HBV) and anti-HCV (antibodies for HCV), respectively, using commercial immunoassays (ELISA). Total alcohol intake was computed according to the average ethanol content of wine (12 percent by volume), beer (5 percent) and spirits (40 percent) and duration of consumption. History of heavy alcohol intake was defined as the consumption of at least 60 g of ethanol per day for at least 10 years.

In order to investigate PCB distribution according to HCC aetiology, three major categories of risk factors were considered, i.e. HCV, HBV and heavy alcohol intake, and each subject with more than one risk factor was assigned to the group with the factor with the highest risk for HCC [11].

Since PCB values showed a skewed, non-normal distribution, the geometric mean, median, range and 90th percentile are reported and the comparison of PCB concentrations among HCC patients according to HCC aetiology was performed using the Kruskal-Wallis non-parametric test. Since each HCC case was matched to one healthy subject (control) by gender and age, a matched analysis was performed. In particular, the non-parametric Wilcoxon test for paired data was used for the comparison between HCC cases and controls for the serum level of total PCB and main PCB congeners.

RESULTS

Most HCC cases were males (78.2%), with a mean age of 68.8 years (SD: 7.4) and a low level of education (83.2% with 3-8 years of education). Most HCC cases were diagnosed by liver biopsy (68%), and the remainder by AFP>500 ng/dl, sonography or computerized tomography. Among the 69 cases of primary liver cancer undergoing biopsy, 2 were mixed HCC and cholangiocarcinoma and the remainder pure HCC. As regards body mass index (BMI), most HCC cases were overweight or obese at diagnosis, having a BMI of 25 and over (55.4%); 27.7% of the HCC cases claimed to have lost 5 or more kg in the 10 years before diagnosis.

Total PCB serum levels of the HCC patients did not vary according to demographic, clinical or epidemiological characteristics, including BMI and weight loss, apart from age (Spearman's correlation coefficient: 0.23; p=0.02). Furthermore, no difference was found between patients who had and those who had not lost 5 or more kg of body weight in the last 10 years (medians of PCB serum level: 1197.0 and 1020.5 ng/g lipid, respectively).

Of the 101 healthy subjects, 79 were males and 22 females, with a mean age of 70.8 years. No statistically significant difference was found in the demographic characteristics of the HCC patients and healthy subjects.

As regards the main risk factors for HCC, 25 patients had HCV infection alone (24.7%), 9 HBV infection alone (8.9%), 33 a history of heavy alcohol intake (at least 60 g/day for at least 10 years) (32.7%), 16 both HCV infection and a history of heavy alcohol intake (15.8%) and 13 cases no risk factor (12.9%). Overall, these three risk factors were found in 88 HCC patients (87%).

TABLE 1

GEOMETRIC MEAN, MEDIAN, RANGE AND 90TH PERCENTILE OF LIPID-ADJUSTED SERUM LEVEL OF TOTAL PCBS ACCORDING TO AETIOLOGY OF HEPATOCELLULAR CARCINOMA IN 101 PATIENTS AND IN 101 HEALTHY SUBJECTS FROM THE GENERAL POPULATION

	SERUM TOTAL PCB VALUES (NG/G LIPID)				
	NO.	GEOMETRIC MEAN	MEDIAN	90TH PERCENTILE	RANGE
ALL HCC PATIENTS	101	1091.2	1081.0	1994.0	287.0-3182.0
RISK FACTORS FOR HCC					
HCV INFECTION*	44	986.8	1021.0	1904.0	287.0-3050.0
HBV INFECTION#	11	1092.2	1271.0	1684.0	382.0-1972.0
HEAVY ALCOHOL INTAKE°	33	1214.9	1128.0	2286.0	510.0-3182.0
AT LEAST ONE RISK FACTOR	88	1077.6	1076.5	1983.0	287.0-3182.0
NONE	13	1166.9	1081.0	2367.0	615.0-2665.0
GENERAL POPULATION	101	1352.8	1199.3	3839.9	225.7-22825.0

PCB = polychlorinated biphenyl; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; HBV = hepatitis B virus.
* HCV infection alone + HCV/HBV co-infection + HCV infection and alcohol intake of 60+ g/day.
# HBV infection alone + HBV infection and also an alcohol intake of 60+ g/day.
° Alcohol intake of 60+ g/day.

Table 1 shows the geometric mean, median, range and 90th percentile of total PCB serum values in HCC patients, overall and according to major risk factors, and in healthy subjects. In HCC patients, no difference was found in total PCB serum levels according to the three main risk factors (Kruskal-Wallis test: p=0.3) or between HCC patients with and without major risk factors for HCC (p=0.6). A statistically significant difference was found in serum total PCB concentration between the HCC cases and the healthy subjects (Wilcoxon test for paired data: p=0.02).

Only 7 of the PCB congeners searched for were detected in the serum of at least 30% of the subjects; they were, in decreasing order of concentration: PCB 180, 153, 138, 170, 194, 118 and 156. A comparison of the median of these congeners and of the PCB TEQ between HCC patients and healthy subjects is shown in Figure 1. Significantly higher values for PCB 118 and for PCB TEQ (close to statistical significance p=0.06) but lower values for all the other PCB congeners were found in HCC patients compared to healthy subjects (p<0.05).

For each PCB congener, no statistically significant difference in serum levels was found in HCC patients according to the major HCC risk factors or between HCC patients with and without major risk factors (p>0.1).

DISCUSSION

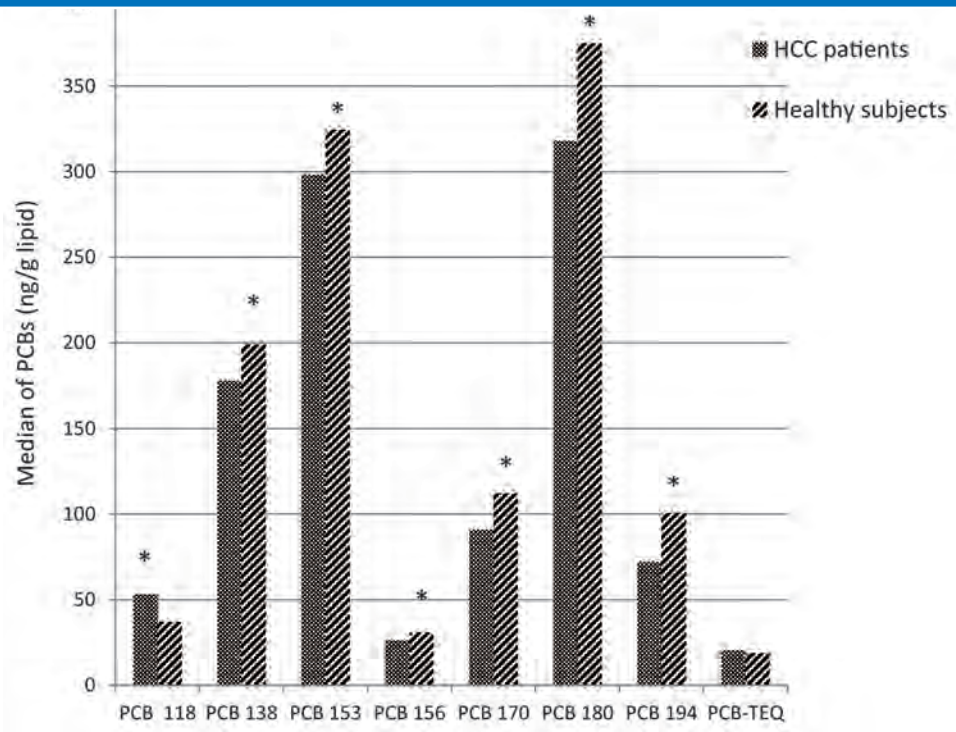
The main finding of this study is the lack of significant differences in total PCB serum concentration in HCC patients with and without, and according to, the major risk factors for the disease, and between HCC patients and healthy subjects living in the area.

Almost 90% of HCC patients had one or more of the main risk factors for HCC, in agreement with previous research in this and other South European areas [10]. The role of the known risk factors for HCC may actually have been underestimated in this study, as we considered a relatively high threshold for heavy alcohol intake (60 g/day) and did not search for occult hepatitis B virus infection [16] or other factors [11]. However, the finding of no differences in PCB serum levels in HCC patients with and without the major risk factors for the disease suggests that the role of these compounds as single agents in HCC development is probably modest, if any, in this area.

The lipid-adjusted serum concentration of

FIGURE 1

MEDIAN OF LIPID-ADJUSTED SCRUM LEVEL OF PCB CONGENERS IN 101 HEALTHY SUBJECTS FROM THE GENERAL POPULATION AND IN 101 HCC PATIENTS



total PCBs was not higher in HCC patients than in healthy individuals living in the area, of the same age and gender as HCC cases. Among the PCB congeners, higher serum levels were found for 118, a DL-PCB with a very low TEF (0.00003), but lower levels for other congeners, in HCC patients compared to healthy people. Although we cannot rule out that the differences found for PCB 118 may be due to chance, it should be noted that it caused cancer of the liver, lung and uterus in female rats exposed to food with high levels of PCB in toxicological studies [5]. In our study, however, PCB 118 was one of the congeners with the lowest serum levels in both HCC patients and healthy subjects, and therefore its contribution to total PCB concentration and PCBTEQ was modest.

Since most HCC patients develop liver cancer after a long history of cirrhosis, some concern may be raised as to whether possible weight loss due to liver disease may have determined a decrease in PCB serum levels in these patients [17]. This seems unlikely to have occurred in our study, however, because we found no difference in PCB serum levels between HCC patients who had and those who had not lost body weight in recent years. Furthermore, although a modified serum/adipose tissue ratio of PCB concentration can be expected in HCC patients, we found a serum/adipose tissue ratio of the medians of total PCB concentrations of 0.5813, very similar to the 0.53 found in healthy pregnant women living in the area [18].

Both HCC patients and healthy people living in the area showed high PCB serum levels compared to those found in other Western countries, confirming that people living in this area had undergone significant PCB exposure, mainly through the consumption of polluted food [7]. Total PCB serum levels were significantly higher in HCC patients than in healthy people and do not support a cause-effect association between PCB exposure and HCC development, in line with occupational cohort studies, most of which failed to show an increase in liver cancer in workers exposed to PCBs [19]. On the contrary, an excess of deaths from liver cancer and chronic liver diseases was found among people who had ingested rice oil contaminated with PCBs and polychlorinated dibenzofurans (PCDFs) in the Yusho (Japan)

and Yucheng (Taiwan) incidents, compared to the national mortality rates [20,21]. However, almost 90% of the total toxic equivalent (TEQ) in the blood of the contaminated people was contributed by PCDFs in both incidents [20,21], raising doubts as to the actual effects of PCBs [22]. This is different from the pattern found in our area, where PCBs contributed more than polychlorinated dibenzo-p-dioxins and dibenzofurans to TEQ serum levels [23].

CONCLUSIONS

This study has some limitations. First of all, the relatively small number of subjects with no known risk factors for HCC did not allow us to draw definite conclusions as to the role PCBs may play in HCC development as single agents. Second, this study had a retrospective design, using PCB serum levels as a biomarker of past exposure. However, PCB serum levels can be considered a reliable measure of PCB body storage, as shown by the high correlation of PCB levels, adjusted for lipid concentration, between serum and adipose tissue (Spearman's r=0.91), serum and liver (r=0.79) and liver and adipose tissue (r=0.75) in patients with a first diagnosis of HCC before they underwent any treatment for the disease [13].

In conclusion, these findings do not support the hypothesis that PCBs play an important role as risk factors for HCC, although a contribution by some specific congeners cannot be ruled out.

ACKNOWLEDGEMENTS: *This study was undertaken with the collaboration of the personnel of the Internal Medicine and General Surgery departments at the Spedali Civili and the S. Orsola Hospital in Brescia, who provided precious help in recruiting the HCC cases. We are grateful to Dr. Bruno Milanesi and to the staff of the Analysis Laboratory of the Desenzano Hospital for performing the virological analyses on the HCC cases.*

References

[1] Lauby-Secretan B, Loomis D, Grosse Y, et al. Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. Lancet Oncol 2013;14:287-8.

[2] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for selected Polychlorinated biphenyls-Update. U.S. Department of Health and Social Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA 2000.

[3] Ludewig G, Robertson LW. Polychlorinated biphenyls (PCBs) as initiating agents in hepatocellular carcinoma. Cancer Lett 2013;334:46-55.

[4] National Toxicology Program NTP toxicology and carcinogenesis studies of 3,3', 4,4', 5-pentachlorobiphenyl (PCB126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (Gavage Studies) NatlToxicolProgram Tech Rep Ser 2006; 520:4-246.

[5] National Toxicology Program NTP toxicology and carcinogenesis studies of 2,3', 4,4', 5-pentachlorobiphenyl (PCB 118) (CAS No. 31508-00-6) in female Harlan Sprague-Dawley rats (Gavage Studies) NatlToxicol Program Tech Rep Ser 2010;559:1-174.

[6] Apostoli P, Magoni M, Bergonzi R, et al. Assessment of reference values for polychlorinated biphenyl concentration in human blood. Chemosphere2005;61:413-21.

[7] Donato F, Magoni M, Bergonzi R, et al. Exposure to polychlorinated biphenyls in residents near a chemical factory in Italy: the food chain as main source of contamination. Chemosphere2006;64:1562-1572.

[8] Brescia Local Health Authority. Available from: http://www.aslbrescia.it/media/documenti/vari/registro_tumori/Pubblicazione_RT_2004_2006.pdf

[9] Donato F, Tagger A, Gelatti U, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol2002;155:323-31.

[10] Donato F, Gelatti U, Limina RM, et al. Southern Europe as an example of interaction between various environmental factors: a systematic review of the epidemiologic evidence. Oncogene2006;25:3756-70.

[11] El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118-27.

[12] McGlynn KA, Abnet CC, Zhang M, et al. Serum concentrations of 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) and1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE) and risk of primary liver cancer. J Natl Cancer Inst 2006;98:1005-10.

[13] Zani C, Gelatti U, Donato F, et al. Polychlorinated biphenyls in serum, liver and adipose tissue of subjects with hepatocellular carcinoma living in a high polluted area. Chemosphere2013;91:194-9.

[14] Van den Berg M, Birnbaum LS, Denison M, et al. The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. ToxicolSci2006;93:223-41.

[15] Phillips DL, Pirkle JL, Burse VW, et al. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ ContamToxicol1989;18:495–500.

[16] Raimondo G, Pollicino T, Romanò L, et al. A 2010 update on occult hepatitis B infection. PatholBiol (Paris)2010;58:254-7.

[17] Lim JS, Son HK, Park SK, et al. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes (Lond) 2011;35:744-7.

[18] Bergonzi R, Specchia C, Dinolfo M, et al. Distribution of persistent organochlorine pollutants in maternal and foetal tissues: data from an Italian polluted urban area. Chemosphere2009;76:747-54

[19] Zani C, Toninelli G, Filisetti B, et al. Polychlorinated biphenyls and cancer: an epidemiological assessment. J Environ Sci Health C Environ CarcinogEcotoxicol Rev2013;31:99-144.

[20] Onozuka D, Yoshimura T, Kaneko S,et al. Mortality after exposure to polychlorinated biphenyls and polychlorinated dibenzofurans: a 40-year follow-up study of Yusho patients. Am J Epidemiol2009;169:86-95.

[21] Tsai PC, Ko YC, Huang W, et al. Increased liver and lupus mortalities in 24-year follow-up of the Taiwanese people highly exposed to polychlorinated biphenyls and dibenzofurans. Sci Total Environ 2007;374:216-22.

[22] Ross G. The public health implications of polychlorinated biphenyls (PCBs) in the environment. Ecotoxicol Environ Saf2004;59:275-91.

[23] Turrio-Baldassarri L, Abate V, Battistelli CL, et al. PCDD/F and PCB in human serum of differently exposed population groups of an Italian city. Chemosphere 2008;73:S228-34.

*