Assessment of intake and related toxicity of polychlorinated biphenyls in the Italian diet.*

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Introduction

The recent Belgian incident has focused the attention of scientists and consumers on the potential health risks related to the intake of dioxins, dibenzofurans and polychlorinated biphenyls (PCB) through the diet, and on the possibility that food contamination by impredictable sources might substantially increase this risk. In this study1 we have measured the intake of PCB with the food in a group of subjects consuming a tipical Italian diet prior of the so called "Belgian incident". Results could be therefore used as a reference to monitor possible changes in PCB background levels in food in Italy introduced by this incident.

PCB are organochlorine compounds used in industry, which are highly persistent in the environment; reports from the literature suggest their toxicity in man, showing that maternal exposure may cause serious neurodevelopmental problems[1] and intellectual impairment in newborns[2] and that PCB may play a role in the etiology of breast cancer[3], non-Hodgkin lymphoma and other lymphatic/hematological malignancies[4]. PCB accumulate in organisms through the food chain and the diet is considered the main source of human exposure[5], with fish and animal fats being the main sources in an average western diet. However, since dietary habits vary greatly among populations, levels and sources of these compounds may differ in different populations.

Materials and methods

1.Sample collection and preparation.

A group of 20 healthy volunteers living in areas around the town of L'Aquila, Italy, were instructed to prepare an extra portion of all the meals and snacks eaten in a day. Foods were pooled in a glass container and stored refrigerated. Immediately after collection, the containers were delivered to the laboratory, where each individual pool was weighed, homogenized, lyophilized and stored at -20°C until analysis. This procedure was repeated twice on non-consecutive days, giving rise to three separate 24-hour collections for each volunteer.

For PCB analysis, 5g portions of the lyophilized sample were spiked with 1 ng of each of the internal standards ($^{13}C_{12}$-labelled PCB congeners numbers 77, 101 and 169) and Soxhlet extracted for 8 hours with n-hexane: acetone (9:1; v/v). The extract was evaporated, the fatty fraction was re-dissolved in ethyl acetate: cyclohexane (1:1; v/v) and PCB were purified by gel permeation chromatography on Bio Beads S-X3. After clean-up with ethyl acetate:cyclohexane (1:1 v/v), PCB were eluted with 100 ml of the same solvent. The solvent was concentrated to about 50 µl. Recoveries of PCB extracted and purified by this method ranged from 60 to 100%, depending on the specific congener considered.

2.PCB analysis.
Analysis were done using a HP 5890 gas chromatograph coupled with a VG TS-250 mass spectrometer equipped with a NB-54 capillary column, 50m x 0.20 mm i.d., 0.33 µm film thickness. The GC program was as follows: 125°C for 2 min, raised 7.5°C/min to 190°C and subsequently 2°C/min to 300°C, maintained for 5 min. Helium was used as carrier gas with head pressure of 200 kPa. The injector temperature was 280°C and the GC-MS interface was held at 280°C. Analysis were done in the selected ion recording mode. Labelled congener 77 $^{13}C_{12}$ was used as internal standard for the tri- and tetra-CB, 101 $^{13}C_{12}$ for the penta-CB and 169 $^{13}C_{12}$ PCB for hexa- and hepta-CB.

3.TCDD-TEQ determination for risk assessment.
A TCDD toxic equivalency (TEQ) value for each PCB congener was calculated by multiplying its content in the 24-h samples by the corresponding toxic equivalence factor (TEF), based on mammalian toxicity data, according to WHO-ECEH (European Centre for Environment and Health) and IPCS (International Programme on Chemical Safety)[6].
For each sample a total TEQ value was calculated as the sum of the individual TEQ of 13 PCB congeners in each sample. The congeners considered were those reported to contribute most to the TCDD-like total toxicity of PCB, according to WHO-ECEH and IPCS[6].

Results
1.PCB intake.
The mean±SD intake of total PCB (mean±SD of the three 24-h determinations), was 3.72±1.51 µg/person/day. Individual levels varied within one order of magnitude, ranging from 0.97 to 10.59 µg/person/day. The figure 1 reports the distribution (mean±SD of the daily intake) of the 30 PCB congeners analysed in foods. Congeners 153, 18 and 138 were found the highest levels (13.8%, 11.4% and 10.9% of the total) whereas coplanar PCB 126, 77 and 169 were globally present as only 0.5% of the total. According to the chlorination class (from tri- to hepta-CB), there was a slight prevalence of the lower chlorinated compounds tri-CBs (28%), with tetra-, penta- and hexa-CB in about the same proportions (20-21% each) and the higher chlorinated compounds hepta-CB in a much lower concentrations (9%).

2.TCDD-TEQ.
The figure 2 reports the individual TEQ values, calculated by multiplying the daily intake by the corresponding TEF for each PCB congener. TEQ are expressed as pg/person/day for each subject during the three experimental days. TEQ for subject 2 (day 3) and subject 19 (day 2) were particularly high, since it was found that their diet contained significant concentrations of congener 126 (6.86 and 15.8 ppt). Excluding these two samples from the calculation, the mean±SD TEQ was 39.5±21.5 pg/person/day. The TEQ values for subject 2 on day 3 and subject 19 on day 2 were 2109 and 4553 pg/person/day.

Discussion

The mean value and range found in this study are consistent with previous estimates for the general population in some European countries. However the exposure to PCB we measured are still higher than the minimal risk level recommended by International Agencies [0.02 µg/kg/day. Agency for Toxic Substances and Disease Registry, Division of Toxicology, Minimal risk levels (MRLs) for hazardous substances, March 1996][7].
To correctly estimate risks associated with PCB, we need to measure exposure to specific PCB congeners, since each of them has particular properties, with characteristic patterns of persistence and toxicity potential. Many of the effects of PCB are similar to those reported for TCDD. Structure-function relationships for PCB congeners have identified two major structural classes of PCB that elicit "TCDD-like" responses, namely the non-*ortho* coplanar PCB (IUPAC 77, 126, 169) and their mono-*ortho* derivatives (IUPAC 105, 114, 118, 123, 156, 157, 167, 189).
These congeners, together with the most representative di-*ortho* congeners (IUPAC 170 and 180) have been considered in order to estimate the toxicity potential of PCB exposure. Their TEF were used to calculate individual "TCDD-like" TEQ values for exposure, expressed as pg/person/day for each subject during the three experimental days. The mean found in this study, 0.57±0.32 pg/kg/day, is well below the estimated minimal risk levels (10 pg/kg/day. WHO/EURO, Consultation on tolerable daily intake from food of PCDDs and PCDFs, Bilthoven 4-7 December 1990, Copenhagen)[8] but occasional exposure to highly toxic congeners may cause this limit to be exceeded. Due to exposure to congener 126, TEQ of 26 and 50 pg/kg/day were recorded in two subjects on two separate occasions. This indicates that PCB contamination of food might well be a risk for humans. Furthermore, since exposure could be potentially affected by the consequences of the so called “Belgian incident”, this study could be useful as a reference to monitor recent changes in PCB background levels in food in Italy.

References

***** Data reported here have been recently published: Zuccato E, Calvarese S, Mariani G, Mangiapan S, Grasso P, Guzzi A, Fanelli R. Chemosphere, 1999, 38 (12): 2753-2765.
1. CJ Yung-Chen, G Yue-Liang, H Chen-Chin, WJ Rogan. JAMA 1992: 268: 3213-3218
2. J.L. Jacobson and S.W. Jacobson. N. Engl. J. Med. 1996; 335: 783-789.
3. M.S. Wolff, P.G. Toniolo. Environ. Health Perspec. 1995; 103 (Suppl 7): 141-145.
4. B.G. Svensson, Z. Mikoczy, U. Stromberg, L. Hagmar. Scand. J. Work Environ. Health 1995; 21: 106-115.
5 R. Duarte-Davidson, K.C. Jones. Sci. Total Environ. 1994; 151:, 131-152.
6 U.G. Ahlborg et al. Chemosphere 1994; 28: 1049-1067.
7 Agency for Toxic Substances and Disease Registry, Division of Toxicology, Minimal risk levels (MRLs) for hazardous substances, March 1996.
8 WHO/EURO, Consultation on tolerable daily intake from food of PCDDs and PCDFs, Bilthoven 4-7 December 1990, Copenhagen.

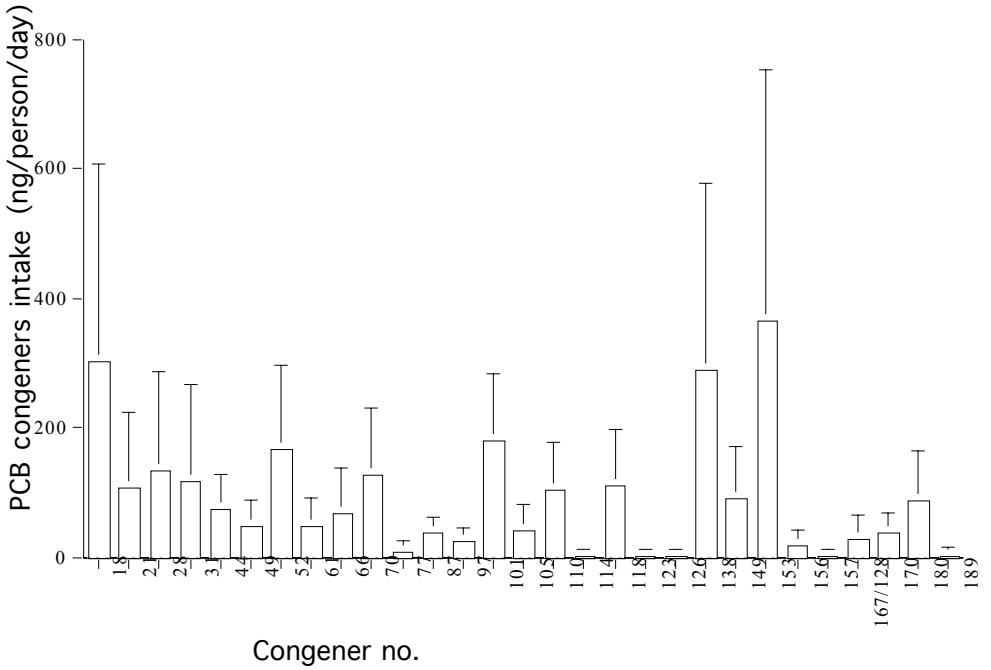

Figure 1. Means±SD dietary intakes of 30 PCB congeners.

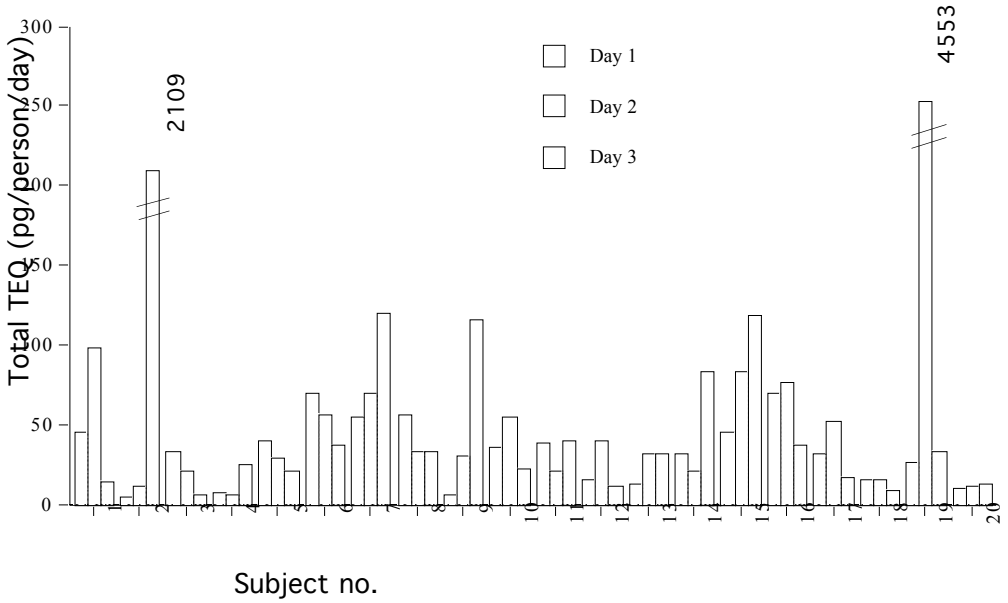

Figure 2. Total toxic equivalency (TEQ) values for each subject during three 24-h periods.