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LEVEL, SOURCES AND TOXICITY OF POLYCHLORINATED BIPHENYLS IN THE ITALIAN DIET

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Abstract

We used the duplicate portion method to measure the daily dietary intake of total and congener-specific polychlorinated biphenyls (PCB) and to assess their potential toxicity in a group of 20 subjects consuming a typical Italian diet.

The mean±SD intake of total PCB, measured by GC-MS, was 3.72±1.51 µg/person/day, comparable to values reported in similar studies world-wide, with individual intakes varying within one order of magnitude, from 0.97 to 10.59 µg/person/day. The di-*ortho* congeners 153, 18 and 138 were the PCB found in the highest concentrations (respectively 13.8%, 11.4% and 10.9% of the total) while the non-*ortho* coplanar congeners (77, 126 and 169) amounted to 0.5% of the total.

The corresponding levels of toxicity (TCDD-like TEQ values ascribable to PCB) ranged from 4.6 up to 119 pg/person/day of TCDD-equivalents in 18 subjects, i.e. presumed no-risk levels, but with peaks of 2109 and 4553 pg/person/day in two subjects with significant intakes of the congener 126.

Principal components analysis and redundancy analysis showed dairy products, meat and fish were the principal sources of PCB, and vegetables those with the highest toxicity index in the Italian diet.

1. Introduction

Polychlorinated biphenyls (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 intellectual impairment in newborns (1) and that PCB may play a role in the etiology of non-Hodgkin lymphoma (2) and other lymphatic/hematological malignancies (3-4); a role in breast cancer has been suggested (5-6) but not always confirmed (7). Some PCB congeners in the environment may

have significant estrogenic activity and might be linked with reproductive and endocrine disruption in fish, birds and mammals, including humans (8,9).

Since PCB accumulate in organisms through the food chain, the diet is considered the main source of human exposure (10), with fish and animal fats being the main sources of PCB in an average western diet. However, since dietary habits vary greatly among populations and PCB are ubiquitous in the environment, levels and main sources ofthese compounds may differ in different populations.

In this study we used the duplicate portion method to measure the daily intake of PCB from food by Italians, to assess toxicity and potential health risks by calculating the exposure to TCDD equivalents (TEQ), and to identify the principal sources of PCB in the Italian diet.

2. Materials and methods

Sample collection

Twenty healthy volunteers (7 males, 13 females; mean age±SD 38±13 years; mean body weight±SD 69.5±12.3 kg) living in rural areas around the town of L'Aquila, Abruzzo, 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 of one of the participating centres, 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, to be subsequently analysed.

Volunteers were also instructed to keep a written record of the type and weight of all the raw ingredients used to prepare meals and of the ready-made foods eaten during each experimental period. Records were processed to obtain the total weight of the edible part for each of the following classes of food items: meat (all animal meats, fats and gelatines, excluding dairy products), dairy products (milk, yogurt, cheese, cream and butter), eggs (fresh or incorporated in cakes, pasta and similar), fish (all kinds of fish and shellfish) oil (olive and other vegetable oils and fats), green vegetables (green leafy vegetables, such as salad, spinach and similar), non-green-leafy vegetables (all others vegetables excluding cereals), cereals (pasta, bread, rice, cakes and others), sweets and sweeteners (sugar, honey and similar), fruits (including juices), alcoholic beverages (wine, beer and spirits), non-alcoholic beverages (water and other drinks, including coffee and tea). The weight of each specific ingredient was calculated using synoptic food composition tables (11).

Analysis of PCB

Throughout this paper the IUPAC numbering system was used to classify PCB congeners. In foods we measured PCB congener numbers 77, 105, 114, 118, 123, 126, 156, 157, 167, 169, 170, 180, 189, because these are reported to be the most toxic, and congener numbers 18, 21, 28, 31, 44, 49, 52, 61, 66, 70, 87, 97, 101, 110, 128, 138, 149, 153 which are those reported to be the most abundant in the environment (12).

Chemicals

All solvents were of pesticide grade (Carlo Erba, Milan, Italy). Bio-Beads S-X3 200-400 mesh were obtained from Bio-Rad Laboratories, Richmond, CA, USA. Anhydrous sodium sulphate was from Merck (Darmstadt, Germany). Glass fiber thimbles and glass fiber filter GF/D were from Whatman International Ltd. (Maidstone, England).

A mixture of PCB congeners numbers 28, 52, 101, 118, 138, 153 and 180 (PCB Mix 3) was obtained from Labor Dr. Ehrenstorfer (Augsburg, Germany). Individual congeners numbers 77, 105, 114, 123, 126, 156, 157, 167, 169, 170, and 189 and $^{13}C_{12}$ labelled congeners numbers 77, 101 and 169 (to be used as internal standards) were obtained from Cambridge Isotopic Laboratories (Andover, Massachusetts, USA). A stock solution containing these 20 PCB congeners and the three internal standards was made up for quantitative analysis.

Aroclor 1232, 1242, 1254 and 1268 and individual congeners numbers 18, 21, 31, 44, 49, 61, 66, 70, 87, 97, 128 and 149 were obtained from Analabs Inc. (North Haven, Connecticut, USA).

Sample preparation

Fifty-g samples (wet weight) of each 24-h pooled food sample were weighed, lyophilized and stored frozen until analysis. Five-g 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) (13). The extraction efficiency for selected samples was calculated to be in the range 96-101%. Samples were subsequently dehydrated on anhydrous sodium sulphate, and filtered on a glass fiber filter. The extract was collected in a pre-weighed round-bottom flask, the solvent was evaporated under vacuum and the weight of the fatty fraction was calculated. The fatty fraction was then re-dissolved in 15 ml of ethyl acetate:cyclohexane (1:1; v/v) and PCB were purified by gel permeation chromatography (GPC) on 45 g of Bio Beads S-X3 (column length 320 mm, internal diameter 25 mm). After clean-up with 130 ml of ethyl acetate:cyclohexane (1:1 v/v), PCB were eluted with 100 ml of the same solvent (14,15).

The solvent was concentrated under vacuum to 5 ml, transferred to a 10-ml centrifuge tube and reduced to about 50 µl under a gentle nitrogen stream. Recoveries of PCB extracted and purified by this method ranged from 60 to 100%, depending on the specific congener considered.

HRGC-MS 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 (Analytical Technology, Cernusco S/N, Milan, Italy). 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.

The MS resolution was 1000 and the electron impact ionisation voltage 30 eV, with a source temperature of 200°C. Analysis were done in the selected ion recording mode: ions M^+ and M^{+2} were recorded for tri-, tetra- and penta-CB and ions M^{+2} and M^{+4} for hexa- and hepta-CB.

PCB congeners were identified by comparison of their retention times with those of the reference standards. A standard mixture of Aroclor 1232, 1242, 1254 and 1268 was prepared and used to evaluate the elution range and the congener distribution of the various chlorinated classes on the mass chromatograms during

instrumental analysis. Isotopic ratios between the areas of two ions recorded for each class were also considered and identification was positive when the isotope ratio was within 15% of the theoretical value.
Quantitative analysis was done by the isotopic dilution method. PCB congener numbers 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 170, 180 and 189 were quantitated by comparison with their respective reference standards.
Since previous studies on the analytical response of a group of 45 PCB congeners to EI-MS evaluated by monitoring their positive molecular ion showed there was little response variability within congener groups (16,17), in this study we used the average molecular ion response, calculated as the average response of the available standards of each group, to quantify the total PCB of each chlorinated class and the PCB congeners 18, 21, 31, 44, 49, 61, 66, 70, 87, 97, 128 and 149. Labelled congener 77 $^{13}C_{12}$ was used as internal standard for the tri- and tetra-CB, labelled congener 101 $^{13}C_{12}$ for the penta-CB and labelled congener 169 $^{13}C_{12}$ PCB for hexa- and hepta-CB.

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) (12).
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 (12).

Multivariate analysis

To identify the principal sources of PCB and of the PCB congeners contributing to the TCDD-like toxicity in the diet, we used multivariate techniques to analyse the 36 samples for which both PCB congener concentrations and synoptic composition of foods were available. Food composition was expressed as the percentage in dry weight of each specific item (except beverages, which were not included in this analysis) for each sample.
A preliminary principal components analysis (PCA) was done to study differences in the concentrations of PCB congeners among samples. PCA determines the closeness of differences among samples on the basis of their multivariate composition (18-19). PCA has limitations, however, in assigning the relative contribution of more than one source to the contaminated sample.
Redundancy analysis (RDA), another multivariate technique with greater power to detect the specific effects of external variables of interest, was used to correlate PCB congener concentrations and their TCDD-like toxic contribution with the synoptic composition of foods. PCA was carried out using the program SCAN, release 1 for Windows (Minitab Inc., State College, USA), RDA with the program CANOCO, version 3.0 (20).

3. Results

PCB intake

Individual dietary intakes of total PCB (mean±SD of the three 24-h determinations), with the mean±SD for the 20 subjects investigated, are reported in figure 1.

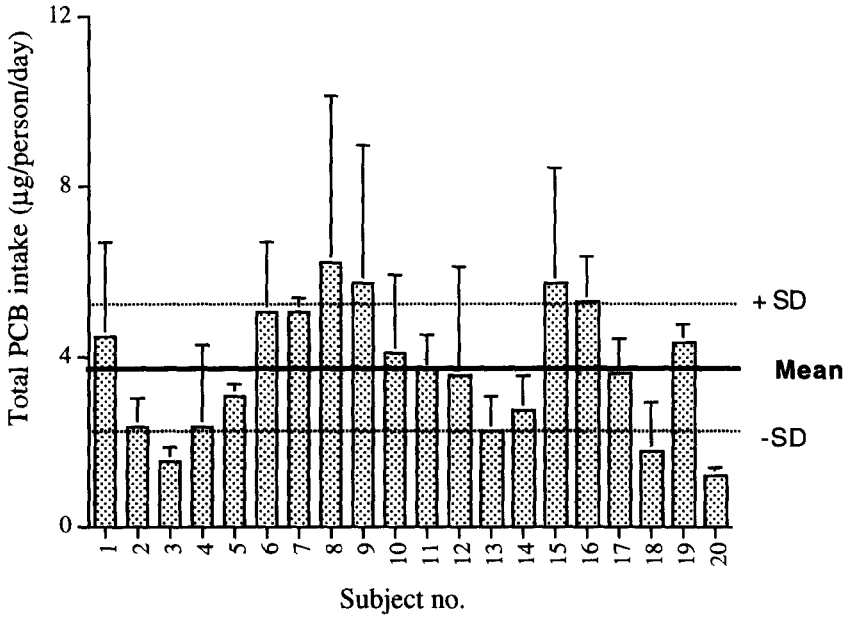

Figure 1. Dietary intake of total polychlorinated biphenyls (PCB). Individual values (mean±SD of three 24-h collections for each subject) and mean±SD for the whole group.

The mean±SD intake was 3.72±1.51 µg/person/day, corresponding to 0.053±0.022 µg/kg/day. Individual levels varied within one order of magnitude, ranging from 0.97 to 10.59 µg/person/day. Figure 2 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.

Figure 3 summarises the distribution of the PCB congeners in food, according to their 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%).

TCDD-TEQ

The individual TEQ values, calculated by multiplying the daily intake by the corresponding TEF for each PCB congener, are shown in figure 4. 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 IUPAC 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 (with individual values ranging from 4.6 up to 119 pg/person/day), giving to a mean of 0.57±0.32 pg/kg/day (range 0.08-1.67 pg/kg/day).

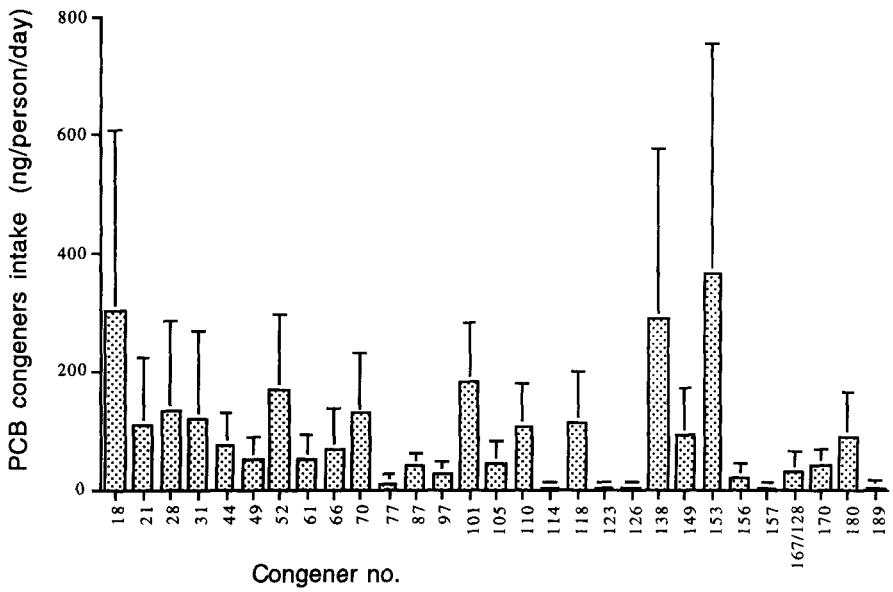

Figure 2. Means±SD dietary intakes of 30 PCB congeners.

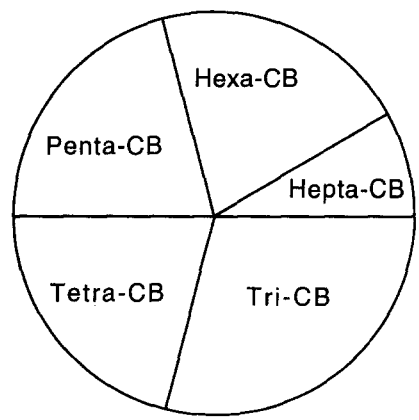

Figure 3. Relative distribution of the PCB congeners present in food grouped according to the chlorination class.

The TEQ values for subject 2 on day 3 and subject 19 on day 2 were 2109 and 4553 pg/person/day, corresponding to respectively 27.4 and 56.9 pg/kg/day.

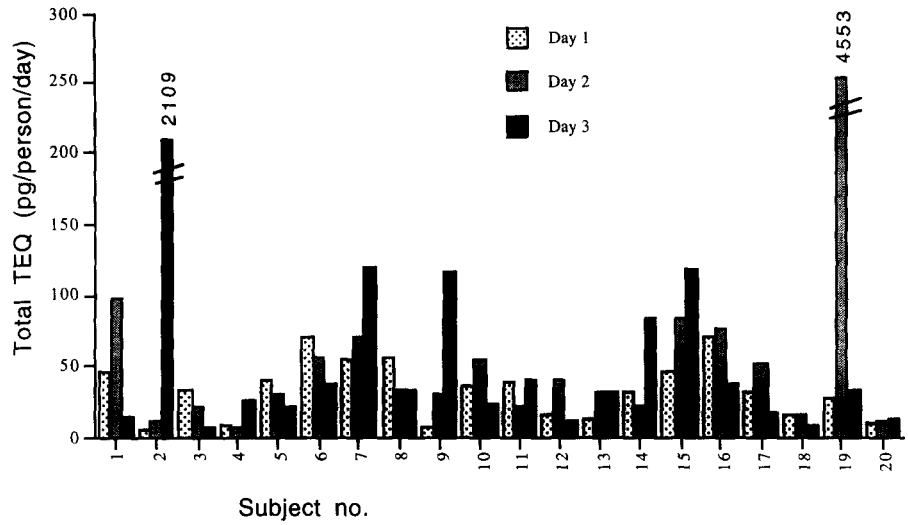

Figure 4. Total toxic equivalency (TEQ) values for each subject during three 24-h collection periods.

Multivariate analysis

PCA indicated that the variability in PCB concentrations could be grossly ascribed to differences in the content of two groups of food items in the diet, vegetables (including green leafy vegetables, other vegetables and vegetable oils) and dairy products (including butter, milk, yogurt, cheese and cream) (figure 5). RDA confirmed that 33.2% of the variability in the PCB concentrations (and in the TEQ due to PCB) among samples could be explained by differences in the content of specific food items in the diet. Figure 6 shows the scatterplot of the samples on the first two components, where the full-line arrows indicate changes in the concentration of the PCB congeners (and of total PCB and TEQs) and the dotted-line arrows changes in the composition of the diet. A part of the variability (20.5 %) is explained by the association of congeners 97, 87, 110, 105, 118, 189, 114, and total PCB, with dairy products, including butter (which was analysed separately).

A smaller proportion of the variability (8.1%) is explained by the correlation between congeners 126, 21, 28, 31, 77, 18, 149, and total TEQ for PCB, with vegetables (non-green and leafy green vegetables) and vegetable oils. Another 4.6% of the variability, indicated by the third axis extracted (not shown in the figure), is explained by the relationship between congeners 167, 138, 157 and 153 and meat and fish.

4. Discussion

The widespread use of PCB and their improper disposal have resulted in their entering the environment. Moreover, the relative stability of the more highly chlorinated PCB and their lipophilicity, mean these compounds are now widely distributed and transported throughout the environment, and their residues have

been identified in air, water, aquatic and marine sediments, human adipose tissue, serum and milk, and in food.

It has been estimated that the majority of human exposure to PCB come from the diet (97% in the UK population) (10) and that the intake of total PCB by the general population depends greatly on the geographical area and food habits. Dietary intakes varying from 0.5 up to 48 μg/day have been reported for different countries. On the bests of available data, an average intake of 5-15 μg/day for the general population in industrialized countries was probably a reasonable estimate in the 80's (21); recent papers from the UK report lower levels of exposure (total PCB intake of 0.53 μg/day in UK) (10).

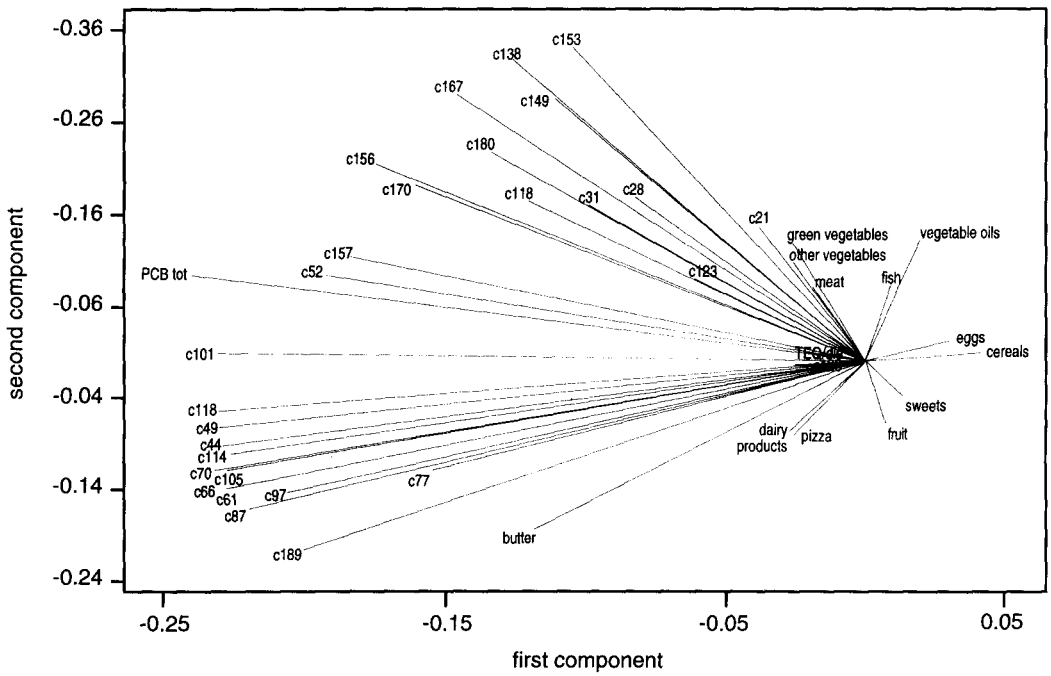

Figure 5. Principal components analysis (PCA) loading plot of the variations of PCB congener concentration and the corresponding differences in food composition among samples.

However, most papers report "estimated" values, calculated from market "basket" studies, i.e. measuring PCB concentrations in selected foods and then multiplying for their estimated intake in the general population. This may introduce several biases since individual intakes of specific foods vary greatly within a population and PCB levels are also affected by food processing, i.e. washing and cooking (22). Moreover, certain items may be excluded from the analysis (10) and this may underestimate PCB intake. The method used in this study overcomes these limitations by measuring PCB in duplicate portions of the food eaten by each member of the study group in 24 hours. It therefore provides experimentally-determined means and limits of the PCB intake, which may be useful to estimate the range of intake by the general population (23).

The mean (3.72±1.51 μg/person/day) and range (0.97-10.59 μg/person/day) found in this study are greater than those recently reported in the UK but are consistent with previous estimates for the general population in some European countries in the 80's (21). Since no previous data are available for Italy, we cannot draw any conclusions but it would appear there has been no reduction in PCB exposure in recent years. The exposure

to PCB we measured (0.053±0.022 μg/kg/day) are still higher than the minimal risk level (0.02 μg/kg/day) recommended by international agencies (24).

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 in the human body and toxicity potential. For instance, the higher CB (hexa- and hepta-CB) have a lower clearance rate than the lower ones, so they remain in the body longer. Congener 28 has an estimated half-life in the human body of three years, no. 105 of 6.7 years, no. 118 of 9.8 years, no. 138 of 16.3 years and no. 153 of 27.5 years (25-26).

Consolini and others (27) reported the most abundant PCB congeners in human adipose tissue from Italian surgical patients were IUPAC numbers 153, 138 and 180. In our study congeners 153 and 138 were first and third in abundance in food, and congener 18 was second. However, congener 18 is a low chlorinated PCB, and is presumably cleared rapidly from the body, so its lack of accumulation in adipose tissue is not surprising. We found congener 180 was the most abundant of the hepta-CB, i.e. a class with high persistence. It seems therefore that there is concordance between the PCB congeners introduced with food and the congeners that accumulate in adipose tissue.

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 (12). 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) (28) 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 and that specific dietary patterns may lead to large increases in PCB exposure and associated health risks. Recent reports do in fact indicate that the maternal exposure to PCB through the diet might be the cause of the intellectual impairment observed in some newborns (1) and that dietary or professional exposure to PCB might play a part in the etiology of breast cancer (5-6), non-Hodgkin lymphoma (2) and other lymphatic/hematological malignancies (3-4).

It has been stated that fish is the main dietary source of PCB, and this may be true in areas such as Japan or North American Great Lakes regions where fish from polluted water may account for a large part of the diet; in Northern European countries, however, the main sources of dietary exposure to PCB were dairy and other animal products (21). However, the source of dietary exposure to PCB for the general population may be greatly affected by the geographical area and food habits. Cereals and vegetables were, for instance, the principal contributors to total dietary PCB in Vietnam and India (29-30).

We found that dietary exposure to total PCB was attributable mainly to dairy products and to a lesser extent to meat and fish, but exposure to the PCB with the highest TEF was principally related to vegetables. Since vegetables were mainly associated with the PCB of the lower chlorination class (tri- tetra-and the lower penta-CB, including isomers with high TEF such as 77 and 126), and fatty foods were related to the highly chlorinated PCB, generally with lower TEF (dairy products with penta-CB, meat and fish with hexa-CB), one interpretation of this observation is that vegetables may be contaminated by atmospheric fall-out, while the selective accumulation of the higher-chlorination class PCB in animal tissues might be the result of their

persistence in living organisms, where they have a longer half-life than PCB of the lower chlorination classes (25-26). Supporting this observation, Duarte-Davidson and Jones (10) reported that the congener profile for air and soil in UK was dominated by the lower-chlorinated compounds.

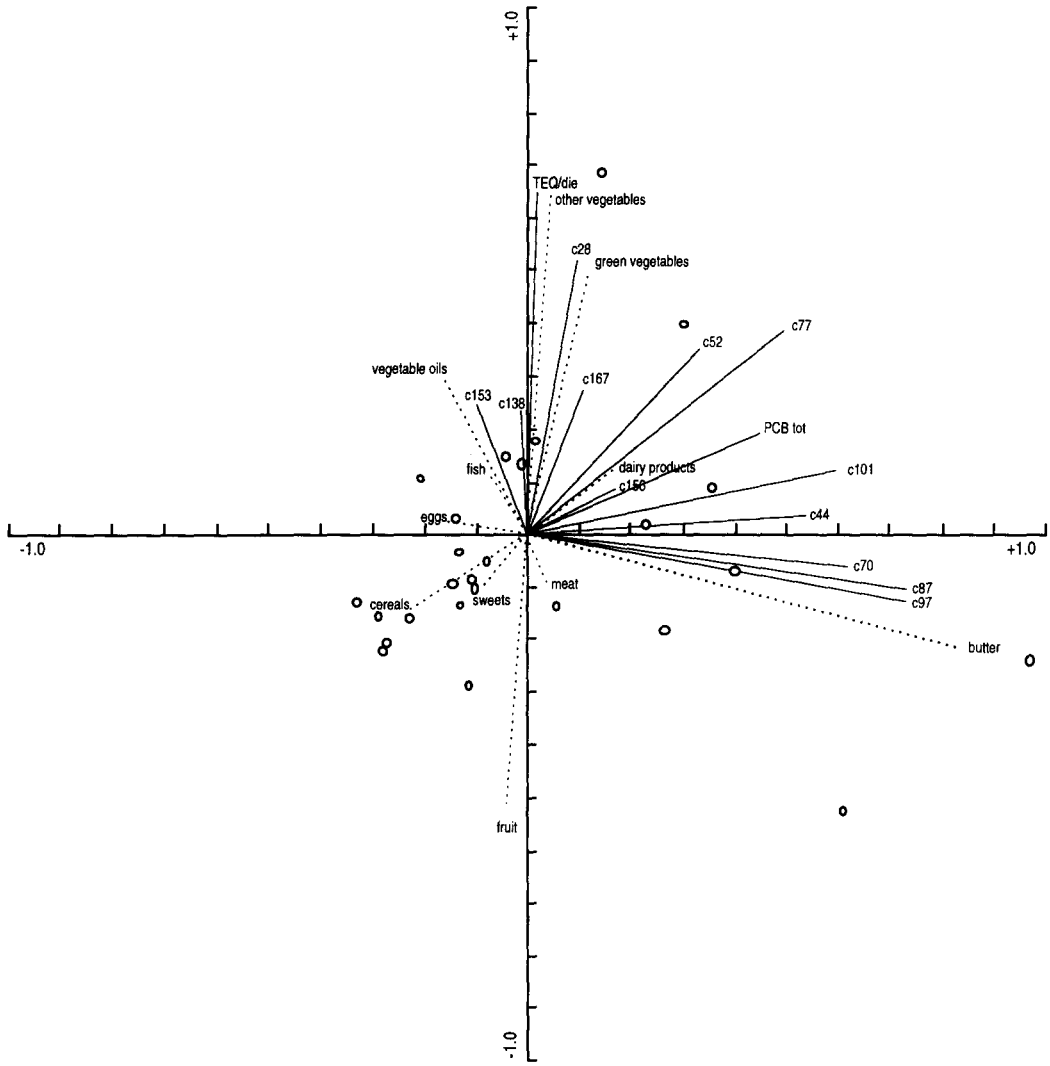

Figure 6. Scatter plot of the redundancy analysis (RDA) showing samples and variation of PCB congener concentrations and of the corresponding differences in food composition among samples.

In conclusion, in this study we used the duplicate portion method to measure the daily alimentary intake of total and congener-specific PCB in a group of subjects eating a typical Italian diet, i.e. a kind of diet for which no previous data were available. The mean daily intakes were comparable to those from other countries

estimated by less specific methods (i.e. market "basket" studies), but the inter-individual sensitivity of this method showed that individual exposure to PCB may vary greatly, both qualitatively and quantitatively. As a result, while the levels of toxicity ascribable to PCB fall into a presumably low-risk category for most of the population, they may be higher in groups accidentally exposed to highly toxic PCB congeners. In our diet, dairy products, meat and fish were the principal sources of PCB, and vegetables contained the most toxic PCB.

5. Acknowledgements

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