# NATIONAL STUDY ON POLYCHLORINATED BIPHENYL BLOOD LEVELS IN FRENCH CONSUMERS OF FRESHWATER FISH (ICAR-PCB) (2): PREDICTORS OF HUMAN BLOOD PCB LEVELS

Desvignes V[1*], Merlo M[1], de Bels F[2], Zeghnoun A[2], Leblanc JC[1], Favrot MC[1], Marchand P[3], Le Bizec B[3], Volatier JL[1]

[1]ANSES (French Agency for Food, Environmental and Occupational Health & Safety, 27-31, avenue du Général Leclerc, F-94701 Maisons-Alfort, [2]InVS (French Institute for Public Health Surveillance), 12, rue du Val d'Osne, F-94415 Saint-Maurice, [3]LUNAM Université, Oniris, LABERCA, Nantes, F-44307, France

**Introduction**

Polychlorinated biphenyls (PCBs) are persistent and bioaccumulative environmental contaminants that have been banned in France since 1987. These chemicals are associated with a wide range of health effects. Due to past uses, these lipophilic substances are still widespread in the environment and in foodstuffs. In the general population, diet represents 90% of PCB exposure, with fish as a major contributor [1]. In December 2006, the European Commission set maximum levels for certain contaminants in foodstuffs, in particular for dioxins (PCDD/Fs) and dioxin-like PCBs (DL-PCBs) in fish[2]. This regulation was updated in December 2011 and completed regarding non-dioxin-like PCBs (NDL-PCBs)[3]. Since the application of these regulations, freshwater fish exceeding maximum regulatory levels have been found in several rivers in France. Could consumption of these fish increase serum PCB levels?

In 2008, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the French Institute for Public Health Surveillance (InVS) set up a national study to investigate the possibility of a statistical relationship between blood PCB levels and the consumption of freshwater fish with a high level of PCB bioaccumulation ("Bio+" freshwater fish as opposed to "Bio-" fish, in the following abstract). This second abstract presents the predictors of blood PCB levels and proposes to predict frequencies of consumption of Bio+ freshwater fish[4] presenting no risk to consumer health.


**Figure 1: The study sites**

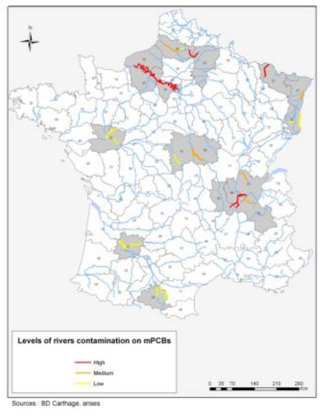


**Figure 2: The study areas**

**Materials and methods**

*Study population*

The population of anglers and their families was sampled from six sites in France (Figure 1) with a complex survey design to investigate the factors that predict PCB body burden. These six rivers or sections of rivers were assumed to include different PCB contamination levels in their sediments: high levels of PCBs (the Seine and the Somme), medium levels (the Rhone and the Rhine-Moselle system) and low levels (the Loire and the Garonne).

People between 18 and 75 years old, living in the six areas (current residence) and holding a fishing license, were eligible to participate. People with occupational or accidental exposure and/or chronic or serious pathologies were excluded from the study. Eligible subjects had to accept to be interviewed and to donate a 50mL whole blood sample. All participants provided written informed consent, and the study protocol was approved by the ethical Committee of Protection of People of Ile-de-France IX Henri Mondor.

*Data collection*

All anglers were interviewed by phone and asked to describe their consumption of Bio+ freshwater fish (eel, barbell, bream, carp, sheatfish and roach)[5]. They described the consumption of these fish for all members of the family (between 18 and 75 years old). Sampled people were surveyed at home about demographic characteristics (age, gender, BMI, etc), about consumption of specific freshwater fish (Bio+ freshwater fish as well as Bio- freshwater fish: bleak, gudgeon, pike, black-bass, crucian carp, chub, hotu, perch, catfish, pike perch, tench, trout, dace, minnow), and finally about general dietary habits. Data were collected with a food frequency questionnaire.

*Blood PCB determination*

Serum was analyzed for seven congeners of marker PCBs (PCB-28, 52, 101, 118, 138, 153 and 180). We calculated the serum total PCB concentration by summing three PCBs (PCB-138, PCB-153 and PCB-180) and multiplying this sum by the factor 1.7. This factor was calculated for the present population (quantification of all PCB congeners for 83 randomly sampled participants). All samples were over the limit of detection (LOD) and all serums were lipid adjusted. PCB body burden is expressed as ng/g lipid.

*Statistical methods*

Considering the low number of members of angler households enrolled and the variability of levels of fish contamination on each river section, the sections were grouped on the basis of fish contamination data in order to increase robustness of analysis and facilitate interpretation of the results. A hierarchical clustering algorithm built three clusters of contamination levels (high, medium and low) as “area” (Figure 2).

We used log-transformation for blood levels, as preliminary investigations indicated that PCB level was consistent with a lognormal distribution (Kolmogorov-Smirnov test).

Potential explanatory factors were identified from the bibliography[6, 7]. They corresponded to demographic and lifestyle characteristics as well as dietary habits (consumption of freshwater fish and sea fish, all foodstuffs containing PCBs, home consumption). To assess the shape of the relationships between the log-transformed serum congener concentrations and the continuous explanatory factors, we used mathematical transformations such as log transformation, square root transformation or a spline regression with three degrees of freedom, when necessary.

We used the regression model to identify significant predictors of natural log concentrations of serum PCB. The model was built in three steps. First, the model contained basic demographic and lifestyle characteristics (age, sex, BMI, etc.). After analysis, we only retained in this model explanatory factors with more than 70% probability of association. Second, the previous model was used to screen for association with other co-variables of consumption. Co-variables were tested singly and adjusted for the model. Again, we only retained explanatory factors with more than 70% probability of association. Third, significant variables were then added to the model for multivariate analysis. Stepwise procedures were applied to select variables that best predicted blood PCB levels in multivariate models. We only retained in the model explanatory factors that were statistically significant ($p<0.1$). The Akaike information criterion (AIC) was examined. To confirm the goodness of fit, we calculated a coefficient of determination ($R^2$) for the model and analyzed the pattern of residuals (normal distribution and homoscedasticity). We examined explanatory factors for collinearity using a correlation matrix of variables, a correlation matrix of model parameters and variance inflation factors. We calculated Cook’s distances and used sensitive analyses to investigate the model’s robustness.

For our analysis we did not take account of the complex survey design, in order to keep a high exposure contrast. Statistical analyses were performed using SAS 9.1 and 9.2 (SAS Institute Inc., Cary, NC) and R 2.12.

**Results and discussion**

*Blood PCB level*

The geometric mean of blood PCB level was 491.1 ng/g lipid (CI 95%, 462.2-523.5) and the 95$^{th}$ percentile was 1461.8 ng/g lipid (Table 1). The PCB-153 congener was used as a marker to compare serum levels across others published studies. Serum levels of the present population were higher than those of the North American population (126.1 ng/g lipid *vs* 19.5 and 24.2 ng/g lipid)[8, 9]. These differences could be explained by higher environmental contamination in France and different dietary habits (in the North American population there is less consumption of fish, which is a major contributor to PCB exposure). Finally, they were within the range of the current French general population (130 ng/g lipid)[10] and have declined since the 1980s (360 ng/g lipid in the general population)[11].

| | **PCB-153 (ng/g lipid)** | **Total PCBs (ng/g lipid)** |
|---|---|---|
| Geometric mean | 118.6 (95% CI, 111.4- 126.3) | 491.9 (95% CI, 462.2- 523.5) |
| Arithmetic mean (SD) | 156.3 (5.0) | 644.5 (20.2) |
| Median | 126.1 | 527.2 |
| 75th percentile | 205.2 | 844.4 |
| 95th percentile | 352.5 | 1461.8 |
| Minimum | 9.8 | 37.8 |
| Maximum | 1022.1 | 4580.3 |

**Table 1: Serum levels of PCB congeners of the participants**

*Regression model*

The regression model explained 66.5% of variation in blood PCB level and showed that a large part of variation was explained by demographic and lifestyle characteristics (63%) unlike consumption factors which explained only a small percentage of variation (Table 2). Some factors were associated with an increased PCB body burden, such as age, current consumption of Bio+ freshwater fish (caught in the study area), gender (male), area (high contamination) and consumption of Bio- freshwater fish (not caught in the study area). Other factors, such as BMI, weight gain, cheese consumption and egg consumption, were negatively associated with blood PCB level. We found a non-monotonic association between milk consumption and PCB body burden. Other factors were not significantly associated with blood PCB level, such as smoking status, socio-professional category, sea fish consumption, meat consumption and home consumption. The two major factors associated with PCB body burden were age ($R^2$=61%) and, to a lesser extent, current consumption of Bio+ freshwater fish ($R^2$=2%). According to our model, between 18 and 44 years of age, blood PCB level increased by 14 ng/g lipid per year of age, while PCB body burden increased by about 26 ng/g lipid per year of age for older people. An interquartile range of 87.7 ng/g lipid was observed for a deviation of the consumption of Bio+ freshwater fish, of 10.3 times per year (446.9 ng/g lipid for non-consumers and 534.6 ng/g lipid for consumers). This corresponded to an increase of 8.5 ng/g lipid per meal of Bio+ freshwater fish.

| | **$R^2$ (%)** |
|---|---|
| Full model | 66.5 |
| Age | 61 |
| BMI | 0.7 |
| Weight gain (more than 5 kg in the last 5 years) | 0.5 |
| Gender | 0.5 |
| Area | 0.3 |
| Current consumption of freshwater fish with a high level of PCB bioaccumulation (fished in the study area) | 2 |
| Cheese consumption | 0.5 |
| Milk consumption | 0.4 |
| Current consumption of freshwater fish with a low level of PCB (not fished on the study area) | 0.3 |
| Egg consumption | 0.3 |

**Table 2: Contribution of variables to adjusted $R^2$ (%)**

*Estimated frequencies of the consumption of freshwater fish with a high level of PCB bioaccumulation*

We wanted to define the frequencies of the consumption of Bio+ freshwater fish that presented no risk to consumer health. In France, in order to interpret the health impact of blood PCB level, two critical thresholds were established by ANSES scientific committee on contaminants: 700 ng/g lipids for pregnant women, women of childbearing age, breastfeeding women and children under 3 years of age, and 1800 ng/g lipids for the rest of the population[12]. We estimated, from the regression model, frequencies of consumption of these fish which did not lead to blood PCB level exceeding the different thresholds. Several scenarios and hypotheses to calculate these frequencies were considered. Only the most conservative scenario is presented here. It corresponded to an individual with the characteristics of populations who had a blood PCB level equal to or higher than the 95th percentile, living in the most contaminated area and with no weight gain during the last 5 years. For this scenario, we considered two groups: female and male (because of low enrollment in women of childbearing age). The predictive frequencies showed that a woman aged 44, currently consuming Bio+ freshwater fish no more than 5 times per year, would not exceed the critical threshold for PCB. For a man aged 60, the estimated tolerable frequency of consuming this fish would be 28 times per year.

| | | Female | Male |
|---|---|---|---|
| **Factors**<br><br>[a] frequency per year<br>[b] number of portions per year | Age | 44 | 60 |
| | BMI | 23.4 | 27.2 |
| | Current consumption of freshwater fish with a low level of PCB bioaccumulation (not fished in the study area)[a] | 10.6 | 17 |
| | Egg consumption[a] | 53.1 | 72.7 |
| | Milk consumption[a] | 123.2 | 256.3 |
| | Cheese consumption[b] | 664.2 | 634.3 |
| | Area | High contamination | High contamination |
| | Weight gain | No | No |
| **Results**:<br>estimated frequency per year | Current consumption of freshwater fish with a high level of PCB bioaccumulation | **5** | **28** |

**Table 3: Predicted maximum frequency of consumption of Bio+ freshwater fish presenting no risk**

The statistical analysis showed a strong positive association between blood PCB levels and age. It was explained by the progressive bioaccumulation of PCBs in the body throughout one's life. There may also be a birth cohort effect because of the decline in environmental PCB contamination since the PCB ban. The model also showed a positive association between blood PCB level and the current consumption of freshwater fish with a high level of PCB bioaccumulation[13]. The interpretation of the results of this study has led to an Opinion issued by ANSES which made recommendations on freshwater fish consumption[14] to protect French consumers.


**Acknowledgements**

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