NATIONAL STUDY ON POLYCHLORINATED BIPHENYL BLOOD LEVELS IN FRENCH CONSUMERS OF FRESHWATER FISH (ICAR-PCB) (1): DESCRIPTION OF THE STUDY POPULATION

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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. 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? American studies in the Great Lakes area, particularly concerned by PCB pollution, highlighted higher serum PCB levels in freshwater fish eaters[4].

In 2008, the French Ministry of Health requested the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the French Institute for Public Health Surveillance (InVS) to 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 first abstract aims to describe the demographic characteristics, the consumption of freshwater fish and blood PCB levels of participants.

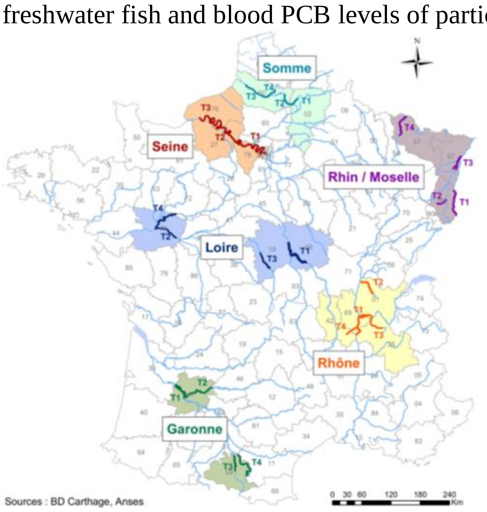

Figure 1: The study sites

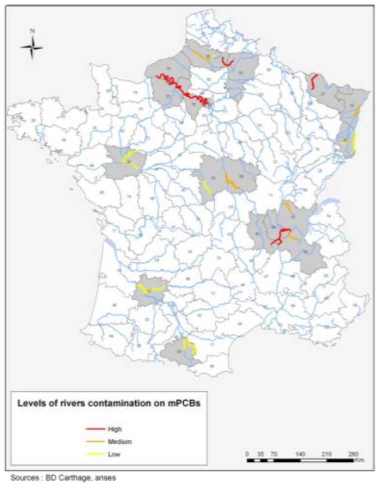

Figure 2: The study areas

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. These six rivers or stretches of rivers represent a total of 900 km and were assumed to include different PCB contamination levels in their sediments: two rivers with high levels of PCBs (the Seine and the

Somme), two rivers with medium levels (the Rhone and the Rhine-Moselle system) and two rivers with 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 (consumers and non-consumers) were then interviewed at home about demographic characteristics, consumption of specific freshwater fish (Bio+ freshwater fish; 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
For analysis in serum samples, a preliminary addition of formic acid was applied, followed by the extraction procedure performed on a C_{18} silica column with two hexane extractions. The total lipid content of the analyzed samples was determined using an enzymatic assay covering four classes of lipids from 50µL aliquots. Further clean-up and separation processes were carried out using classic liquid-solid adsorption chromatography with silica, Florisil and CarbopackC/Celite. The solvents used for the elution were hexane and toluene. An external standard was added for the recovery calculation. According to the isotopic dilution method used for quantification, six ^{13}C-labeled marker PCB standards were added to each sample before extraction. GC-HRMS measurement was performed on an Agilent 7890A gas chromatograph equipped with a DB-5MS capillary column (30 m, Ø 0.25 mm, 0.25 µm) and coupled to a Jeol JMS 800D high-resolution mass spectrometer, at a resolution of 10,000 (10% valley) in selected ion-monitoring (SIM) mode using electronic ionization (EI).
The serum total PCB concentration in the study population was calculated by summing three PCBs (PCB-138, PCB-153 and PCB-180) and multiplying this sum by the factor 1.7. PCB body burden is expressed as ng/g lipid.

Statistical methods
Response rate was the ratio of the number of participants divided by the number of random sampled persons included in the study and contactable by phone.
PCB serum concentrations were described by geometric mean, percentile and range. They were calculated by incorporating the complex survey sample design (clustering and sampling weight).
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 was used to build three clusters of contamination level (high, medium and low) as “area” (Figure 2).

Results and discussion
Participation
The participants were 606 anglers or members of their families, representing 21,180 angler households. Response rate was 44% which is satisfactory for this kind of study and varied according to site, between 31.9% and 49.6% with a maximum for the Rhône site where the environmental PCB pollution is a matter of great concern to fishermen and local authorities. Response rate also varied according to the status of freshwater fish consumption. Consumers participated more than non-consumers (51.6% *vs* 37.8%), probably because they felt more concerned.

Demographic characteristics
The population had a mean age of 44.8 years (CI 95%, 43.4-46.3) and 61.4% were men. The proportions of males and females were roughly similar for each age category (Table 1). Mean body mass index (BMI) of the population was 25.5 (CI 95%, 25.0-26.1) and more than half of the population was thin to medium. The

population was predominantly non-smoker. The major occupational categories of the population were the working class, followed by retirees.

Table 1: Proportions of age categories by gender

Percentage		Gender	
		Male	**Female**
Age	18-44 years	52.9 (45.1-60.7)	51.6 (41.2-62.0)
	45 years and more	47.1 (39.3-54.9)	48.4 (38.0-58.8)
	Total	100	100

Table 2: Proportion of people by area

Area	Percentage	95% CI
High contamination	39.4	(33.2-45.7)
Medium contamination	21.9	(16.7-27.1)
Low contamination	38.7	(32.6-44.8)

The proportion of the population varied from area to area (Table 2) and was lower in areas with medium contamination.

Freshwater fish consumption

The yearly freshwater fish consumption of the study population was on average infrequent (13.0; CI 95%, 11.2-14.8). Only five percent of the study population ate these fish once a week. The study population consumed preferentially Bio- freshwater fish (trout, perch, chub, etc.).

About 13% of the population ate Bio+ freshwater fish (more than twice a year). These consumers ate these fish more than once a month (14.8 times/year; CI 95%, 11.3-18.4). Their consumption of Bio- freshwater fish was two to three times per month (29 times/year; CI 95%, 25.3-32.6). Like the study population as a whole, they consumed more Bio- freshwater fish. The proportion of consumers of Bio+ freshwater fish was similar for both genders (11.4% for female *vs* 14.8 for male), meaning that they had similar behavior concerning fish consumption. The consumers of Bio+ freshwater fish were older than non-consumers (52.6 *vs* 43.6 years old, p<0.0001), they were of stout build (BMI: 27.1 *vs* 25.3, p=0.0001) and lived mainly in low contamination areas.

Dietary habits

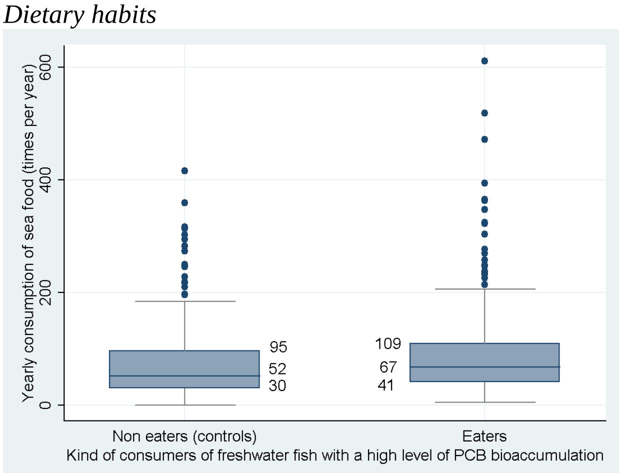

Figure 3: Description of seafood consumption according to the kind of consumers of freshwater fish with a high level of PCB bioaccumulation

The study population ate seafood on average 1.5 times per week. This was less than in the population of the French dietary survey[6] (INCA2 study) (more than twice per week), because in our study we focused on sea species with a high level of PCB bioaccumulation. Consumers and non-consumers of Bio+ freshwater fish consumed similar amounts of seafood. There was no substitution phenomenon of fish eaten (Figure 3). In this respect, regardless of the general dietary habit, our study population was quite similar to the general French population. A few small differences appeared but these were due to the manner in which data was collected: in this study a food frequency questionnaire was used whereas a food consumption record covering 7 days was used for the INCA2 study.

Blood PCB level in humans

For the study population, the geometric mean of blood total PCB level was 399.1 ng/g lipid (CI 95%, 374.6-425.3) (Table 3). For women of childbearing age (between 18 and 44 years old), the geometric mean was 238.9 ng/g lipid (CI 95%, 203.6-280.4). This blood PCB level was lower than that of the study population, probably because of fewer years of exposure. A birth cohort effect might also be supposed. Since the banning of PCBs in France in 1987, environmental pollution has progressively declined and human exposure also. Only 2.5% of the population were over French critical PCB body burden thresholds[7] (700 ng/g lipid for women in childbearing age, 1800 ng/g lipid for the other people).

The PCB-153 median was used as a marker to compare blood PCB levels across studies. Serum levels of the study population were higher than those of the North American population (101.5 ng/g lipid *vs* 19.5 and 24.2 ng/g lipid)[8, 9]. These differences could be explained by higher contamination in France and different dietary habits; in North America, there was less consumption of fish, which is a major contributor in France to PCB exposure. Conversely, blood PCB levels were lower than those in the Czech Republic, suspected of being highly contaminated by PCBs (438 ng/g lipid)[10]. Finally, they were within the range of the current general French population [11] (130 ng/g lipid) and have declined since the 1980s (360 ng/g lipid)[12]. The same trends were observed for women of childbearing age[10, 13].

Body burdens (ng/g lipid)		PCB-138	PCB-153	PCB-180	Total PCB
Study population	**Geometric mean**	43.6 (41.0-46.4)	95.5 (89.6-101.8)	92.4 (86.3-98.9)	399.1 (374.6-425.3)
	Median	47.6	101.5	98.6	411.6
	95th percentile	158.5	328.3	312.0	1355.7
Women of childbearing age	**Geometric mean**	30.2 (25.9-35.1)	59.2 (50.7-69.2)	49.9 (41.9-59.6)	238.9 (203.6-280.4)
	Median	33.9	76.0	63.6	310.2
	95th percentile	71.6	131.3	126.9	585.3

Table 3: Blood PCB levels in the study population and women of childbearing age

We observed that people with the highest blood PCB levels were mostly consumers of Bio+ freshwater fish and were older than the mean for the study population. These observations were confirmed by the statistical analysis, which showed a positive association between blood PCB levels and “age” and the consumption of freshwater fish with a high level of PCB bioaccumulation[14, 15]. The interpretation of the results of this study has led to an Opinion issued by ANSES which made recommendations on freshwater fish consumption[16].

Acknowledgements
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