FOOD EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS AMONG FRENCH HIGH SEAFOOD CONSUMERS (CALIPSO STUDY)

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Introduction

Mainly due to their lipophilic nature, persistent organic pollutants (POPs) accumulate in food chains and have potentially multiple effects on human health including genotoxicity, embryotoxicity and endocrine disruptions. Fish and seafood are major contributors to dietary exposure to persistent organic pollutants: 25% to 30% for the 17 congeners of dioxin and furan type[1 2] (PCDD/Fs), 75% for PCBs (from the indicator PCB (i-PCB)) and 30% for the 7 PBDE congeners (28, 47, 99, 100, 153, 154, 183)[3]. In France, data from the INCA survey (French food consumptions survey) point to an estimated daily dietary intake of PCDD/Fs in adults of 1.45 pg TEQ_{WHO}/kg bw in 2000[1], and 0.5 pg/kg bw in 2006[2]. The daily intake of dioxin-like PCBs (DL-PCBs) was estimated to be 1.2 pg/kg bw in 2006[2].
The objective of the CALIPSO study was to assess accurately food exposure of high consumers of seafood to POPs in order to be able to give appropriate scientific advice or guidelines (e.g. fish consumption recommendations) to consumers considering the toxicological reference values fixed by national and international Authorities like Provisional Tolerable Daily Intake (PTDI) or Weekly (PTWI) or Monthly (PTMI).

Materials and methods

Protocol: Seafood consumption for 80 products of 996 high consumers aged 18 and more in 4 French coastal areas (Le Havre, Lorient, La Rochelle and Toulon) was assessed using a food frequency questionnaire (FFQ). Seafood samples were collected in each region considering ways of buying (fresh, frozen, canned...) and supply habits (fishing or catching, fish shop...) according to a total diet study approach sampling. Food samples were analysed for total lipids, dioxin congeners, furans, DL-PCBs, i-PCBs and PBDEs. Total lipids intake and POPs exposure were assessed by crossing consumption data with contamination data.

Analytic methods: Samples were first lyophilised then ground. For all the analyses, ^{13}C labelled congeners were added before extraction for quantification according to the isotopic dilution method. The lipidic fraction was then extracted by accelerated solvent extraction (ASE) using a toluene/acetone mixture under high pressure and temperature (P=100 bar, T=120°C)[4]. Solvents were evaporated in order to determine the amount of fat. The extract was finally purified in three successive open chromatographic columns. After these fat extraction and purification steps, a quantification standard was added in order to evaluate the recovery yields.
The four fractions obtained corresponding to each of the pollutant classes, are analysed by gas phase chromatography coupled to high-resolution mass spectrometry (GC-HRMS).

Results and discussion

The fish species found to be most contaminated by PCDD/Fs and DL-PCBs were eel with 88.3 pg TEQ_{WHO}/g fresh weight (FW) and fresh sardine with 10.6 pg TEQ_{WHO}/g FW (Data not shown). They are also the most heavily contaminated by i-PCBs, with respectively 2.26 and 117 ng/g FW. Excluding eel, fish presented PBDEs contamination levels (PBDE 28, 47, 99, 100, 153, 154, 183) lower than 3 ng/g. This level increases with the fat content: mackerel, anchovy, seabass, sardine and salmon all have moderately high contaminations between 2 and 3 ng/g fresh weight. Concerning molluscs and crustaceans, swimcrab and crab display the highest levels of PCDD/Fs (18.6 pg TEQ_{WHO}/g FW and crab with 6.5 pg TEQ_{WHO}/g FW respectively) and i-PCB as well, with respectively 187 and 58 ng/g FW. Spider crab displays the highest level of PBDEs with 3.0 ng/g FW. Molluscs are less contaminated whatever the considered POP.

The PCDD/Fs, DL-PCBs and i-PCBs contaminations of seafood products are comparable to those measured by the monitoring plans of French Administrations[5] and the United Kingdom data[6]. PCDD/Fs and DL-PCBs contaminations are within the interval reported by the European Food Safety Authority (EFSA) in 2005: between 0.3 and 5.8 pg TEQ/g FW[7], except in the case of the very highly contaminated eel sample coming from the Netherlands and some crustaceans rarely consumed, such as swimcrab. Concerning PBDEs, our results are consistent with the JECFA data in 2005 on fish and seafood products[10]. Excepting eel, sardine and swimcrab, the samples have levels lower than the regulatory limits (4 pg TEQ_{WHO}/g FW for PCDD/Fs and 8 pg TEQ_{WHO}/g FW for the total of the PCDD/Fs and DL-PCBs)[8]. There is no regulation concerning i-PCBs and PBDEs levels in fish and seafood products.
Generally speaking, the contamination by persistent organic pollutants of our fish and seafood samples displays a north-south gradient (Table 1) although these differences are not statistically significant (on all the products and on the 19 common fish sampled in the 4 zones).

Table 1: Mean concentration of persistent organic pollutants in fish (excluding eel), molluscs and crustaceans per site and standard deviation

	Food samples (Nb samples[a])		Lipids (g/100 g)	PCDD/F (pg TEQ_{OMS}/ FW)	PCB-DL (pg TEQ_{OMS}/ g FW)	Total PCDD/F and PCB-DL (pg TEQ_{OMS}/ g FW)	iPCB (ng/g FW)	PBDE (ng/g FW)
Le Havre	Fish (22)	Mean	3.74	0.36	1.57	1.93	20.5	1.31
		SD	4.78	0.63	3.58	4.18	48.9	1.46
	Mollusc, Crustacean (10)	Mean	2.70	1.44	3.71	5.15	55.0	0.62
		SD	2.13	2.46	7.85	10.3	115	0.51
Lorient	Fish (27)	Mean	3.24	0.34	1.28	1.62	14.6	0.85
		SD	4.85	0.65	2.37	2.98	26.8	0.75
	Mollusc, Crustacean (11)	Mean	2.56	0.73	0.88	1.61	5.97	0.70
		SD	2.62	1.01	1.23	2.22	7.80	0.83
La Rochelle	Fish (23)	Mean	3.19	0.34	1.20	1.53	13.8	0.88
		SD	4.21	0.47	1.84	2.28	20.6	0.68
	Mollusc, Crustacean (12)	Mean	1.78	0.31	0.23	0.54	3.01	0.34
		SD	1.54	0.40	0.22	0.57	3.26	0.14
Toulon	Fish (23)	Mean	3.91	0.25	0.88	1.13	12.2	0.81
		SD	4.73	0.29	0.99	1.24	14.6	0.63
	Mollusc, Crustacean (10)	Mean	1.22	0.16	0.23	0.39	2.06	0.32
		SD	0.69	0.21	0.23	0.43	1.65	0.10

FW: fresh weight. a: Nb composite samples, each sample being made up of 5 sub-samples of the same specie, representative of the supply methods in each zone (port, market, supermarket…).

Table 2 shows that concerning PCDD/Fs subjects have a mean exposure of 4.34 ± 4.25 pg TEQ_{WHO}/kg bw/week and 14.3 ± 15.7 pg TEQ_{WHO}/kg bw/week for PCB-DLs. Only some subjects have a mean exposure to PCDD/Fs and DL-PCBs lower than the WHO's PTMI of 70 pg TEQ_{WHO}/kg bw/month. However 62% of the subjects have an exposure through their seafood consumptions lower than the PTMI. Clearly the average is strongly influenced by certain high values; the statistical distribution is not symmetric. Concerning i-PCBs, the average exposure through their seafood consumptions is 0.40 µg/kg bw/week.
39% of the individuals exceed the PTMI of 70 pg TEQWHO/kg bw/month fixed by the JECFA for PCDD/Fs and DL-PCBs, and 72% of them exceed the TDI of 0.02 µg/kg bw/day fixed by the WHO for i-PCBs. We must remember that other foods also contribute to the intake of PCDD/Fs, DL-PCBs and i-PCBs and that

consequently the total exposures are higher. Our study confirms that even when consuming fish and seafood that comply with the European maximum contamination limits, a high consumer can exceed the toxicological reference values, a fact that has already been shown by other studies[9]. This demonstrates the need to make an effort to reach target values lower than the regulatory limits as rapidly as possible, which is what the new European regulation proposes.

The average exposure to PBDEs (28, 47, 99, 100, 153, 154, 183) is 2.17 ± 1.78 ng/kg bw/day (Table 2). The main contributors to POPs exposure are oily fish sardine: salmon, seabass, mackerel, seabream, cod and tuna (Data not shown). To date no PTWI has been fixed for PBDEs at national, European or international level. But the average exposure to PBDEs, all zones and all subjects included, is consistent with exposures recently estimated in other countries. Total Diet Studies (TDS) published in several countries (Canada, USA, Finland, Netherlands, Spain, Sweden, United Kingdom and Japan) report average exposures of 13 to 228 ng PBDE/day[10]. The levels found in our population of high fish consumers range from 139 to 161 ng PBDE/day with an average of 150 ng/day. Our results are therefore very consistent with those of other studies using similar methodologies. Applying a deterministic exposure model using the data from the French national consumption survey, our contamination data for fish and seafood and data from other product groups contributing to PBDEs exposure taken from European studies, calculation yields an estimated PBDEs exposure of about 63 to 142 ng PBDE/day for the French population compared to 172 to 250 ng/day for our study population. In its evaluation in 2005, the JECFA concluded that the observed exposure of the general population is estimated to be about 4 ng/kg bw/day, which corresponds to 240 ng/day for a person weighing 60 kg, or slightly more than our calculated exposure. This result is very consistent since the JECFA estimation was not based on fish consumption alone. The JECFA considered that in view of the consequent range of exposure for a non-genotoxic compound, the current intakes do not appear to be a cause for concern as regards public health[10].

Table 2 also shows that for all the classes of pollutant a similar trend is observed: the subjects living in Toulon are less exposed than those in the other zones. This is concomitant with the lower POPs contaminations measured in the Toulon samples. This trend is significant for PCDD/Fs and DL-PCBs ($p<0.05$). For the i-PCBs, subjects in both Toulon and Lorient have a significantly lower exposure than those in the other zones. Finally, the average exposure to PBDEs is significantly less in Toulon than in La Rochelle ($p<0.05$), but it is equivalent to that in the two other zones. Globally for food exposure we find a north-south gradient like the one observed for seafood contamination by POPs.

Table 2: Food exposure to persistent organic pollutants of high fish and seafood consumers of all areas regardless of the age and sex (Mean ± SD)

	Le Havre n=249	Lorient n=247	La Rochelle n=248	Toulon n=252	All subjects n=996
PCDD/F (pg TEQ_{OMS}/kg bw/week)	5.58 ± 6.01 ^{a}	4.84 ± 3.79 ^{a}	4.65 ± 3.63 ^{a}	2.37 ± 1.73 ^{b}	4.34 ± 4.25
PCB-DL (pg TEQ_{OMS}/kg bw/week)	17.7 ± 19.3 ^{a}	14.7 ± 13.0 ^{a}	16.1 ± 18.4 ^{a}	8.96 ± 8.59 ^{b}	14.3 ± 15.7
Total PCDD/F and PCB-DL (pg TEQ_{OMS}/kg bw/week)	23.3 ± 25.2 ^{a}	19.5 ± 16.6 ^{a}	20.8 ± 21.1 ^{a}	11.3 ± 10.1 ^{b}	18.7± 19.6
iPCB (µg/kg bw/week)	0.53 ± 0.58 ^{a}	0.35 ± 0.35 ^{b}	0.49 ± 0.77 ^{a}	0.26 ± 0.33 ^{b}	0.40 ± 0.55
PBDE (ng/kg bw/d)	2.23 ± 1.70 ^{a,b}	2.14 ± 1.50 ^{a,b}	2.45 ± 2.31 ^{a}	1.86 ± 1.44 ^{b}	2.17 ± 1.78

Values in the same row with different superscript letters are significantly different, $p<0.05$ (Tukey's test)

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