Factors (trophic levels, fish specie, habitat, fat content…) influencing PCDD/F, PCB and PBDE concentration in fish retailed in France

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INTRODUCTION

Food surveys and exposure studies conducted over the past years have always suggested that the dietary intake of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were due to contamination of food of animal origin and mainly due to fish and fish products consumption. In order to evaluate the exposure of high fish consumers in France an important study has been initiated in 2005.

In this study, 4 sites were selected as sampling areas. The four coastal zones chosen for the seafood consumption survey were Toulon (Mediterranean Sea), Le Havre (North Sea), Lorient (North Atlantic Ocean), and La Rochelle (Atlantic Ocean). The populations in these regions are high consumers of fish and seafood, as confirmed by a Food Consumption Observatory study in 1996. In this study, fillets from different species of fish were purchased from local markets and supermarkets in those 4 areas. The samples were tested for the seventeen 2,3,7,8 chlorosubstituted PCDD/Fs, the twelve "Dioxin-like" PCBs, seven markers PCBs and the seven markers PBDEs. The study presents results for 140 samples from 30 species of fishes and 17 species of molluscs and crustaceans and tries to bring out correlations between contaminants levels and different factors such as: migration, fat content, food diet, habitat, specie or sampling area.

MATERIAL AND METHODS

Sampling

The sampling was dedicated to fishes and seafood mainly consumed by the studied population, considering the form of purchase (fresh, frozen, canned, etc.) and supplying (bought or self-procured). However in this study seasonal effects were not taken into account because the sampling was performed between January and April 2005. In the present case, the analyses were made on raw samples conserved at -20°C in suitable containers until their analysis. This storage temperature was maintained throughout the transportation of the samples to our laboratory.

Samples

A sample of about 1,000 g was made for each fresh product with five 200 g sub-samples. The origin and distribution of these sub-samples were determined according to criteria such as the place of purchase, the consumption frequency and quantity consumed taken from the consumption survey. The 5 sub-samples were mixed, ground to obtain a single homogeneous composite sample of the product. The sub-samples were made up only of the comestible parts of the products. More precisely, fish were filleted and skinned; for shellfish only the soft content was ground (plus the coral in the case of scallops); crustaceans were peeled in order to sample only the flesh (notably legs and claws of crabs and lobsters); mollusc and crustacean samples were made up of raw and/or cooked sub-samples.

Analysis

All the organic solvents (Promochem) were of Picograde® quality. Silica (Fluka), sodium (Merck), acetic acid and sulfuric acid (SDS) were of superior analytical quality. Native and ^{13}C-labeled standards were purchased from CIL. Each sample was homogenated, weighed and freeze-dried. 10-20 g were transferred in Dionex ASE 300 cells. Pressure and temperature were set to 100 bars and 120°C respectively. Basically, the extraction

solvent was a toluene/acetone, 70:30 (v/v) mixture, and three successive extraction cycles (5 min each) were performed. The extract was evaporated to dryness, permitting the estimation of the fat weight. A three steps purification was performed, using successively silica, Florisil and celite/carbon columns. After removal of fat on a silica gel column loaded with sulfuric acid, PCBs were separated from PCDDs/PCDFs by means of a Florisil column. The PCDD/PCDF fraction was further cleaned up onto a column consisting of a mixture of Carbopack C/Celite 545. Separation of coplanar (non-ortho) PCBs from non-planar PCBs was achieved on an activated mixture of Florisil/ Carbopack C/Celite 545 (overnight at 130°C). After addition of external standards for the recovery calculation ($^{13}C_{12}$-1,2,3,4-TCDD for the PCDD/Fs, $^{13}C_{12}$-PCB #111 for the PCBs), the final sample extract was evaporated to dryness under a nitrogen stream and reconstituted by addition of 10 µL of toluene for the PCDD/Fs, 20 µL of toluene for coplanar PCBs and 50 µL of toluene for non-planar PCBs. The GC-HR-MS detection was performed on a HP 6890 gas chromatograph, equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 µm film thickness), and coupled to a Jeol JMS-700D high-resolution mass spectrometer. The injection volume was 2 µL.

RESULTS AND DISCUSSION

A sample of eel was not taken into consideration because of its very high level of contamination. The concentration of this sample was about 88.3 pg TEQ_{WHO} /g FW for the total TEQ, mainly due to "dioxin-like" PCBs with a concentration of 86.8 pg TEQ_{WHO} /g FW. The level of contamination for marker PCBs was at the µg magnitude instead of ng for all the others samples.

Results according to species

Only the fish results will be discussed.

Fish	Lipids (%)	PCDD/F TEQ	DL-PCB TEQ	Total TEQ	m-PCB ng/g FW	m-PBDE ng/g FW
Anchovy	10.8	0.10	0.67	0.77	8.90	2.24
Angler fish	0.33	0.03	0.08	0.11	1.67	0.46
Cats hark	0.88	0.03	0.08	0.10	2.38	0.27
Cod	0.52	0.03	0.11	0.14	1.19	0.54
Common dab	1.02	0.21	0.34	0.55	2.61	0.59
Emperor	6.42	1.44	5.58	7.02	56.4	1.21
Goatfish	4.25	0.54	2.07	2.61	18.8	0.74
Grenadier / hoki	0.59	0.08	0.09	0.17	2.83	0.52
Gurnard	1.15	0.49	1.11	1.60	13.3	0.51
Haddock	0.37	0.07	0.21	0.28	2.74	0.64
Hake	0.96	0.04	0.26	0.30	3.36	0.49
Halibut	12.5	0.89	1.37	2.27	15.0	1.59
John dory	0.91	0.08	0.41	0.50	5.99	0.51
Ling*	0.44	0.04	0.11	0.15	1.75	0.49
Mackerel	7.93	0.60	2.20	2.80	34.5	2.71
Plaice	0.52	0.24	0.53	0.77	6.47	0.63
Pollack	0.30	0.02	0.23	0.25	3.26	0.41
Pout	0.43	0.05	0.18	0.23	1.95	0.42
Ray	1.17	0.09	0.13	0.22	1.52	0.43
Saithe / coalfish	1.43	0.02	0.10	0.12	1.08	0.75
Salmon	13.5	0.50	1.32	1.82	14.5	2.55
Sardine	5.64	1.80	8.77	10.6	117	2.10
Scorpionfish	3.39	0.47	1.74	2.20	16.0	0.60
Seabass	3.70	0.64	3.22	3.86	37.8	2.39
Sea bream	5.49	0.38	2.20	2.58	26.9	1.10
Sole	0.50	0.05	0.15	0.21	4.91	0.39
Swordfish	13.8	0.09	0.43	0.52	4.23	0.85
Tuna	1.02	0.04	0.35	0.39	3.88	0.56
Whiting	0.42	0.05	0.24	0.29	4.26	0.54

Tab1: results for all the fish species. TEQ are expressed in TEQ_{WHO} pg/g fresh weight

PCDD/Fs and DL-PCBs: Table 1 shows that the fishes the most contaminated by dioxins (PCDD/Fs) and dioxin-like polychlorobiphenyls (DL-PCBs) are sardines (10.6 pg TEQ_{WHO}/g fresh weight). They are followed

by the predators emperor fish and seabass with levels of 7.0 to 3.9 pg TEQ_{WHO}/g fresh weight. The least contaminated fishes are catshark, anglerfish, saithe and cod with less than 0.15 pg TEQ_{WHO}/g fresh weight.

m-PCBs: Sardine is also the fish the most contaminated by "markers" PCBs (m-PCBs), with a concentration of 117 ng/g fresh weight. For other species representative of the PCB contamination, we find emperor, seabass and seabream in which the m-PCB levels exceed 30 ng/g fresh weight. The lower contaminated fishes are saithe and cod with values of 1.1 and 1.2 ng/g fresh weight respectively.

m-PBDEs: The fish the most contaminated by polybromodiphenylethers (PBDE 28, 47, 99, 100, 153, 154, 183) is eel (not presented) with an average of 26.6 ng/g fresh weight. The other fishes show contamination levels under 3 ng/g fresh weight. The PBDE level increases with the fat content: mackerel, anchovy, sea bass, sardines and salmon have moderately heavy contaminations, between 2 and 3 ng/g fresh weight. The least contaminated fish is catshark with 0.3 ng/g fresh weight and less than 1% of lipids.

Others results

In order to find other factors which could have an effect on the level of contamination, 3 graphs (numbered graph 1, graph 2 and graph 3) are presented.

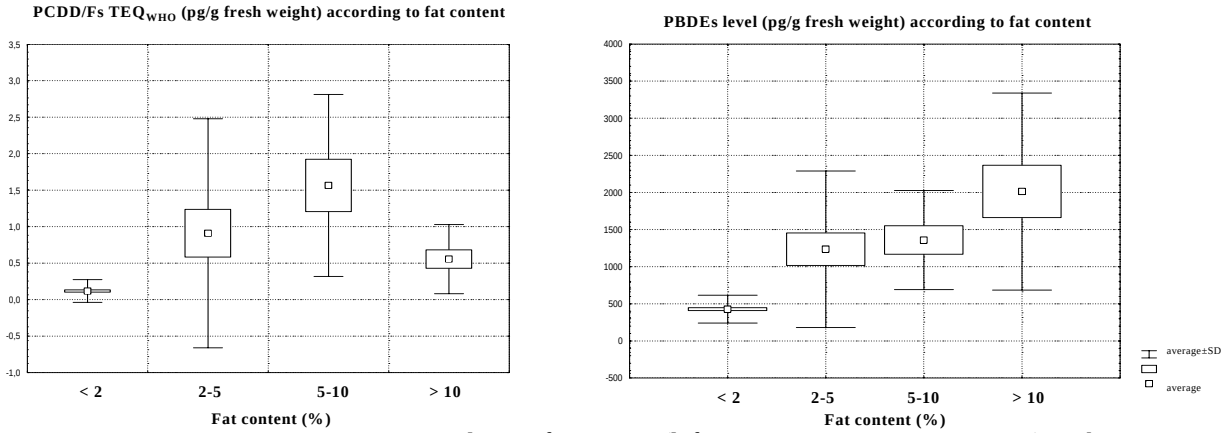

Figure 1: contaminant concentrations according to fat content (left: PCDD/Fs TEQ_{WHO} (pg/g FW); right: PBDEs concentration (pg/g FW))

As we could have expected, the level of the contaminants depends on the fat content of the fish samples. For PBDEs, the level of contamination is found higher (Figure 1) for the fattier samples. For PCDD/Fs and PCBs, the observation is quite different: the TEQ value increases until the fat content reaches 10%. But for very fatty samples (> 10% fat) the levels assessed were not the most elevated.

Another factor studied was the influence of the trophic level on the contaminants concentrations. Three trophic levels are represented by the fish, mollusc and crustacean species of the study: level 2 (herbivores), level 3 (omnivores), level 4 (carnivores). It is known that the persistent organic pollutants concentrations magnify along the dietary chain. This is confirmed by the PBDEs graph (Figure 2). However, it seems, on the contrary, that the PCDD/F and PCB levels decrease when the trophic level increases. But this must be toned down by the fact that we study average concentrations. When we look at standard deviations (SD) we can observe that the SD for herbivore's level is very important whereas carnivore's level has short amplitude. The average observation is not sufficient to describe the results. While for PBDEs, the SD amplitudes are steady for the three trophic levels.
When considering the sampling area, the contamination by persistent organic pollutants of our fish and seafood samples displays a north-south gradient (Figure 3). The Le Havre samples are the most contaminated, for all the pollutants considered, and the Toulon samples are the least contaminated. In Le Havre, the average PCDD/F contamination is 0.71 pg TEQ_{WHO}/g fresh weight, 2.26 pg TEQ_{WHO}/g fresh weight for DL-PCBs and m-PCB contamination reaches 31.63 ng/g fresh weight. The corresponding average PBDE contamination is 1.09 ng/g of

fresh weight. The samples in Toulon are globally the least contaminated with POPs with average PCDD/F and DL-PCB levels of 0.22 pg TEQ_{WHO}/g fresh weight and 0.68 pg TEQ_{WHO}/g fresh weight, respectively. The average m-PCB contamination is 3.14 ng/g fresh weight while the average PBDE contaminations of the Toulon samples are again the lowest: 0.66 ng/g fresh weight. However, we note that these averages are not calculated for the same species in the four regions, but for species that in each region cover about 90% of the fish and seafood consumption of high consumers.

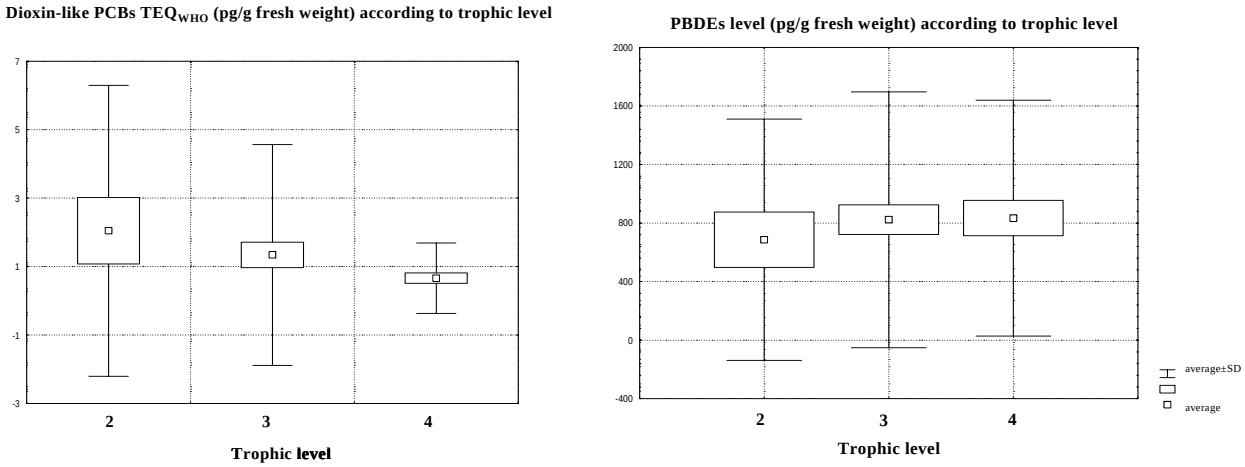

Figure 2: contaminant concentrations according to trophic level (left: PCBs TEQ_{WHO} (pg/g FW); right: PBDEs concentration (pg/g FW))

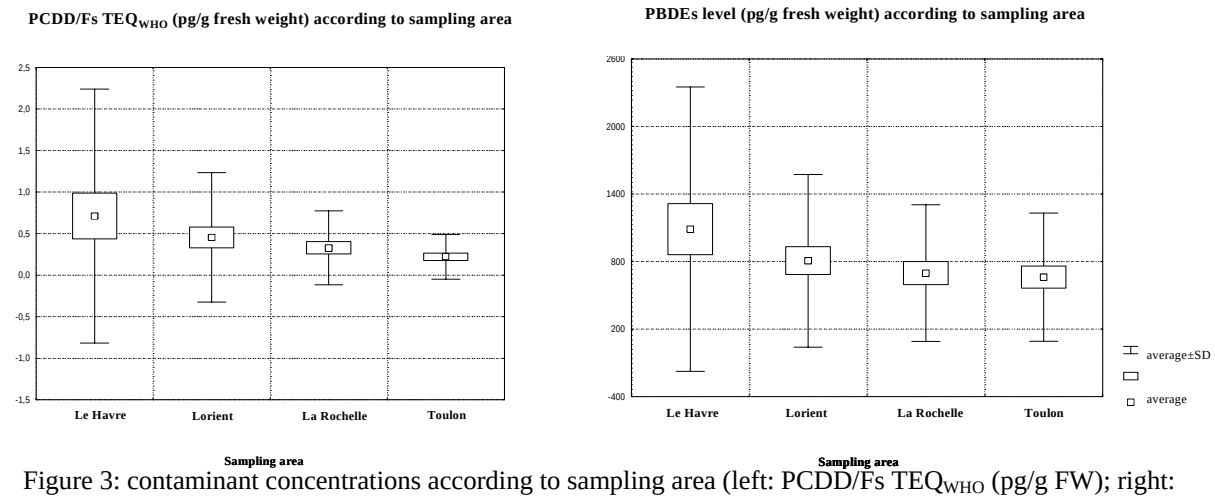

Figure 3: contaminant concentrations according to sampling area (left: PCDD/Fs TEQ_{WHO} (pg/g FW); right: PBDEs concentration (pg/g FW))

Conclusions

A lot of factors must be considered to give some explanations for the contamination results obtained in this study: fat content, trophic level, sampling area, migration, habitat… Some correlations are clearly identified between pollutants levels and studied factors.

Acknowledgements

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