PCBS, PCDD/FS, PBDES AND PAHS IN FISH AND SHELLFISH FROM THE NORMANDY COAST (Eastern Channel, France). PRELIMINARY RESULTS

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Abstract

Dioxins (7 PCDDs and 10 PCDFs -2378 substituted congeners), 12 Dioxin like-PCBs, 7 indicator PCBs, 7 PBDEs and 20 individual PAHs were analyzed in 40 samples of mussels and fish from the Normandy coast in order to cover the whole range of contamination in edible organisms living in French coastal waters. These first results demonstrate that the contamination is generally below maximum concentrations set by the current regulations except for PCBs (DL-PCBs) in a few samples obtained from the estuarine area due to an historical contamination which has been trapped in superficial sediments for many years. Shellfish catching is prohibited in this area and contamination trends in mussels have steadily decreased for the last twenty years. Favorably, PBDE levels are lower than reported previously in mussels in other similar areas in North America or in Europe. Very interestingly, the comparison of contaminant concentrations between mussels and fish shows that some of these substances are partly biotransformed by higher species. However a high consumption of species from the most contaminated areas could lead to a large exposure to contaminants (PCBs). Recommendations are required in order to limit the shellfish consumption from such polluted areas.

Introduction

Seafood may contribute importantly to the human exposure to persistent, bioaccumulable and toxic substances like PCBs, PCDD/Fs, PBDEs and PAHs. Mussels accumulate contaminants from water and from suspended particulate material by filtering large volumes of water and are generally considered as being unable to metabolize contaminants. Fish take up chemical substances from water and mostly from their food and can partly biotransform and eliminate contaminants. Both fish and mussels represent an important part of the general population's diet and consequently they might contribute significantly to the human exposure to contaminants.

Indicator PCBs and PAHs are commonly monitored along the French coasts using bivalve mollusks as sentinel species. Very high PCB and PAH levels are currently measured in mussels collected near the Seine estuary due to the urbanization and industrialization of the whole river basin whereas on the contrary the west coast of the Cotentin Peninsula appears among the cleanest areas along the French coastline. Compared to indicator PCBs and PAHs, data on dioxin-like PCBs, dioxins (PCDD/Fs) and PBDEs are rather scarce. [1]

This study brings new information on these persistent contaminants in various fish species and in mussels collected in three different areas in Normandy in order to describe the whole range of contaminant concentrations in edible species from the French coastal waters. The objectives of the work were to categorize contaminants according to their bioaccumulation or biotransformation capacities by comparing the contaminant patterns in fish to those in bivalves and to estimate human exposure to contaminants due to seafood consumption.

Material and Methods

Sampling

Blue mussels (*Mytilus edulis*) were collected in winter and autumn 2006 in various sampling sites close to the Seine estuary (Area 1), within the Seine Bay (Area 2) and on the west coast of the Cotentin Peninsula (Area 3). Fishes of commercial sizes were obtained from professional fishermen from Le Havre and Octeville (Area 1), from Cherbourg (Area 2) and Granville (Area 3). Species were dissected; composite samples were obtained by pooling together flesh from about 200 mussels or fillets from 3-5 individual fishes.

Analysis

Each sample was homogenated, weighed and freeze-dried. 10 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 step purification was performed. After removal of fat on a silica gel column loaded with sulfuric acid, PCBs and PBDEs were separated from PCDD/Fs by means of a Florisil column. The PCDD/F 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 and PBDEs 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, $^{13}C_{12}$-PCB111 for the PCBs, $^{13}C_{12}$-PBDE139 for the PBDEs), the final extract was reconstituted by addition of toluene in the 3 fractions. 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. For PAHs, after freeze drying, 1 g of the dry residue was taken and spiked with a ^{13}C-labelled internal standard. The samples were extracted according to a selective extraction step using Pressurized Liquid Extraction followed by a purification with polystyrene-divinylbenzene SPE. Identification and quantification were performed using GC-MS/MS, with an isotope dilution approach using ^{13}C-labelled PAHs. For each batch of samples, a quality standard (fish oil) and an analytical blank were systematically added. The sensitivity of the methods developed for all the contaminant families allowed to detect most of the congeners.[2]

Results and Discussion

Levels and distribution of contaminants in mussels and fish

Contaminant concentrations are given for the various groups of compounds in mussels (tab.1) and fish (tab.2) from the various sampling areas arranged together in three groups according to their proximity to the river mouth: 1 close to the estuary, 2 the Bay of Seine and 3 the west coast of the Cotentin peninsula.

Table 1: Contaminant in mussels (mean concentration on a wet weight basis ± 1 standard deviation)

	TEQ PCDD/Fs ng kg^{-1}	TEQ DL-PCBs ng kg^{-1}	Total TEQ ng kg^{-1}	Sum Ind. PCBs µg kg^{-1}	Sum PBDEs µg kg^{-1}	Sum PAHs µg kg^{-1}
Area 1: Seine estuary Villerville (Nb: 4)	1.90±0.54	7.69±2.09	9.59±2.62	156±46	0.95±0.15	44.9±6.5
Area 2: Bay of Seine (Nb: 4)	0.54±0.18	1.03±0.35	1.57±0.50	10.9±4.9	0.18±0.02	5.64±3.76
Area 3: West of Cotentin (Nb: 9)	0.19±0.03	0.29±0.08	0.48±0.10	2.23±0.47	0.17±0.04	5.33±1.71

In all samples, dioxin levels ($TEQ_{WHO\text{-}PCDD/Fs}$) are well below 4 ng.kg^{-1} w. w., which is the maximum level in fish and fishery products according to the European regulation (OJEU, EC N°199/2006). For the total TEQ ($TEQ_{WHO\text{-}PCDD/Fs}$ + $TEQ_{WHO\text{-}DL\text{-}PCBs}$) the maximum level (8 ng.kg^{-1} w. w.) is exceeded in mussels from area 1 and in fish from area 1 and 2 due to the continuous contamination of the Seine estuary by PCBs. Mussel fishing in the estuarine zone is prohibited for safety reasons, as the area suffers from chemical and microbial pollution. Nevertheless, the PCB contamination may reach very high levels in larger fish due to the bioaccumulation and biomagnification of these persistent compounds in foodwebs. The concentrations of PBDEs, expressed as the sum (BDE28, 47, 99, 100, 153, and 154), are around or below a few µg.kg^{-1} w.w. in mussels and fishes, approximately 1-2 order of magnitude lower than "indicator" PCBs depending on the origin of the samples. The PBDE levels measured at Villerville, close to the estuary, are lower than previous measurements in mussels from this same location.[3] These measurements seem to confirm a possible

decreasing trend of this type of contamination. For PAHs, the concentrations (Sum PAHs, the 15 individual compounds according to EU regulations) reach 50 µg.kg^{-1} w.w. in mussels from the estuary, below the recommended maximum levels set at 2500 µg.kg^{-1} w.w. in mussels and 250 µg.kg^{-1} w.w. in fish (AFSSA) [4]. They are one order of magnitude lower in bivalves from other sampling areas whereas in fish samples most of individual PAHs are around or below the levels of quantification.

The results obtained in mussels, the common sentinel species in coastal waters, clearly indicate that the River Seine is the source of contamination that influences the whole bay. The PCB contamination of the Seine estuary and its surroundings has been pointed for a long time. A steep gradient is observed from the estuary to the bay for all groups of contaminants, but to a greater extent for PCBs: ratios of contamination levels in area 1 to that in area 2 vary from 15 for indicator PCBs, around 8-10 for DL-PCBs (TEQ) and PAHs and around 5 for PBDEs. PAHs and PBDEs are present at similar levels in areas 2 and 3, distant from the estuary; that could be due to the very diffuse origin of these groups of environmental contaminants compared to PCBs which are mainly carried by the River Seine into the bay. The relatively high contribution of DL-PCBs to the total TEQ, and the importance of the contribution of PCDFs compared to that of PCDDs are other indications of the influence of the PCB contamination of the River Seine.

Table 2: Contaminant in fish (mean concentration on a wet weight basis ± 1 standard deviation)

	TEQ PCDD/Fs ng kg^{-1}	TEQ DL-PCBs ng kg^{-1}	Total TEQ ng kg^{-1}	Sum Ind. PCBs µg kg^{-1}	Sum PBDEs µg kg^{-1}	Sum PAHs µg kg^{-1}
Area 1						
Seabass *(Dicentrarchus labrax)* Nb = 2	1.4 ; 2.1	9.2 ; 23.4	10.6 ; 25.4	119 ; 537	3.2 ; 3.6	0.17 ; 0.36
Dover sole *(Solea solea)* Nb = 3	0.9±0.2	7.0±1.7	7.9+±1.8	201+±29	0.50±0.10	0.25±0.01
Plaice *(Pleuronectes platessa)* Nb = 4	1.0 ± 0.3	6.2±3.9	7.2±4.1	139±96	0.74±0.50	0.22±0.01
Area 2						
Seabass *(Dicentrarchus labrax)* Nb = 2	0.5 ; 0.8	2.5 ; 8.0	3.0 ; 8.8	24.2 ; 111	2.3 ; 5.2	NA
Dover sole *(Solea solea)* Nb = 1	0.19	0.91	1.10	26.0	0.1	NA
Plaice *(Pleuronectes platessa)* Nb = 2	0.6 ; 0.8	1.3 ; 1.7	1.9 ; 2.4	13.1 ; 16.6	0.18 ; 0.24	NA
Area 3						
Dover sole *(Solea solea)* Nb = 4	0.04±0.14	0.46±0.76	0.5±0.8	20±36	0.07±0.05	0.06
Plaice *(Pleuronectes platessa)* Nb = 4	0.13±0.07	0.32±0.17	0.5±0.2	2.7±1.5	0.09±0.04	0.05

Indeed, the detailed examination of the contaminants congener distribution in samples provides an indication on the processes acting on the distribution and fate of contaminants[5]. The PCCD/F fingerprints differ according to the metabolizing capacities of species; on a concentration basis, main congeners in mussels are OCDD > 2378-TCDF > 1234678-HpCDD > 23478-PeCDF whereas in fish they follow the order: 2378-TCDF > 23478-PeCDF > OCDD > 12378-PCDF which would indicate a more efficient biotransformation of chloro-dioxins than of chloro-furans. PCBs, indicator PCBs and DL-PCBs, present a very similar distribution both in mussels and in fish. Amongst PBDEs, as commonly observed, BDE 47 is by far the main component; BDE99/BDE100 ratios differ between mussels and fishes. Considering that the decabromo mixture is mainly used, the presence of lower brominated congeners, 4-5 Br atoms per molecule, is a sign of complex processes implying debromination of these compounds in the environment and in biota. PAHs are well known compounds which are readily biotransformed into polar compounds and eliminated. The distribution of individual PAHs in fish reflects specific transformation rates according to the structure of the individual components as the general PAH patterns change from mussel to fish.

Bioaccumulation and biotransformation

The distribution of the various individual contaminants of each group of chemicals in fish was compared to that in mussels. In order to categorize these compounds according to their fate in foodweb an approach based on the metabolic slope concept was used [6-8]. For that purpose, an index was calculated according to: MI (metabolization index) = (X_{fish}/$CB153_{fish}$) / (X_{mussel}/$CB153_{mussel}$). Taking into account that food is the main or the only source of contamination, the concentration of each contaminant X was normalized to that of CB153 (22'44'55' hexa-CB) considered as the typical persistent and biomagnified compounds. The comparison between mussels and fish does not reflect the actual foodwebs. In this simplified food chain, at the lower end, mussels take up contaminants from the abiotic environment and do not metabolize them

whereas at the upper end, fish species are contaminated by their successive preys and biotransformation has occurred during their trophic transport to fish or in the predatory fishes themselves. The results are given (tab.3).

Tab 3: Bioaccumulation or biotransformation capacities of contaminants.

MI	
>0.5	CB180 and 153 (MI ref : 1), most of PCBs (DL and indicator PCBs), BDE154
>0.1 and <0.5	CB77 and 81, BDE47,153,183
>0.05 and <0.1	some PCDFs and PCDDs (penta and hexa)
>0.01 and <0.05	PBDE99, OCDD , OCDF, phenanthrene
<0.01	Most of PAHs (BaP MI = 0.005)

The more the ratio MI is beyond or close to one, the more those compounds behave like CB153 and tend to bioaccumulation, unlike PAHs and other compounds with a MI well below (MI<0.03) which are biotransformed and eliminated. The biotransformation of PAHs occurs in higher organisms via the formation of polar arene-oxydes which might also be carcinogenic precursors. Larger molecules with more than 4-5 cycles (BaP, DBaA) are the most metabolized. PCDDs and PCDFs behave intermediately, being more or less biotransformed. Again, PBDEs do not follow the general trend. Surprisingly, BDE47 falls in the group of biotransformed compounds whereas it is the most present amongst brominated compounds. Several points should be investigated in this attempt to categorize contaminants like PBDEs; their presence at very low levels that produce larger uncertainties when calculating concentration ratios, the diffuse characteristic of brominated fire retardants which is not in full agreement with the hypothesis of food as the only source of contamination of fish or a consequence of the debromination of heavier compounds.

Human exposure

The seafood quality is of great health concern as contamination by toxic compounds might reduce its high nutritional value, notably due to its high content of poly-unsaturated fatty acids. The range of the human exposure to contaminants was calculated for adults, taking into account the concentrations obtained here and two scenarios of seafood consumption corresponding to those of mean French consumers (5 g. mussels and 30g fish a day) and five times more for very high consumers. Situations at risk correspond to the worst realistic situations, in which high consumers would regularly eat the most contaminated species from the most polluted areas.

Tab 4:Human exposure to contaminants from seafood calculated from these data.

	PTMI*	**Concentration Range (min-Max)**	**Monthly dietary intake (mean-Max) in weight unit and in PTMI eq. amount**
PCDD/Fs (TEQ)	2100 pg	Mussels: 0.2 - 2 $ng.kg^{-1}$ Fish: 0.05 - 2 $ng.kg^{-1}$	75 - 10800 pg (0.036-5.14 PTMI)
PCDD/Fs and PCBs (total TEQ)	4200 pg	M.: 0.5 - 10 $ng.kg^{-1}$ F.: 0.5 - 25 $ng.kg^{-1}$	525 - 120000 pg (0.125 – 28.6 PTMI)
PAHs	Not relevant	M.: 5 - 50 $\mu g.kg^{-1}$ F.: 0.05 - 0.25 $\mu g.kg^{-1}$	795 - 38625 ng
PBDEs	Not yet established	M.: 0.15 - 1.10 $\mu g.kg^{-1}$ F.: 0.05 - 3.50 $\mu g.kg^{-1}$	675 - 16575 ng

PTMI Provisional tolerable monthly intake for an adult (mean body weight : 60 kg)

These estimations (Tab.4) should be considered to describe an extreme range of variation of the dietary intake from seafood consumption only. Other types of food, meat and dairy products, also contribute to contaminant exposures. These extreme values are greatly influenced by a few specimens who are very contaminated. Larger exposure evaluations do not reflect the actual situation and more probably correspond to a very high consumer who would daily eat 25 g mussels and 150 g fish taken in the most polluted areas. The actual situation is probably comparable with a lower value, which is also in agreement with such estimations in other countries. Nevertheless those data plead for a better assessment of contaminant exposure requiring more detailed investigations.

An attempt in that direction could be done by studying a few key species amongst the most commonly bought and consumed. Compared to food safety monitoring programs, for which the sampling strategy relies upon larger fish supply centers, this study focused on selected areas enabling to obtain data on background situations as well as hot spots of contamination. As far as health is concerned, the consumption of contaminated species, flatfishes, crustaceans and shellfishes from known polluted areas should be maintained within a safer range. For these reasons, regular actions and recommendations should be prescribed in order to limit seafood consumption from such areas.

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