

## Levels and trends of organohalogen compounds in mussels from the Seine estuary in 1981 - 2003

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### 1. Introduction

Even though the use of many organohalogen compounds have decreased or even ceased during the recent years, the release of these contaminants to the environment from different sources continues<sup>1,2</sup>. The research efforts of the last two decades provided evidence of the spread throughout the global environment of certain persistent organic compounds and quantitative understanding of the mechanisms of the distribution of these and other compounds in the environmental reservoirs, including living organisms and human population<sup>3,4</sup>. It appears that these compounds are now embedded in the natural biogeochemical cycles. The recent data reveal also pulses of banned POPs in air concentrations in U.S. and Canada<sup>5</sup>. Furthermore, the inventory of a global cumulative usage of PCBs shows also that usage of these chemicals were highest in western central Europe (ex. France, Benelux, UK and Germany), in eastern U.S. and in Japan<sup>6</sup>, and that in these countries significant amounts are still deposited on various sites. Finally, it has been found, that certain of the “novel” contaminants are organohalogens (brominated, fluorinated chemicals) and might be indeed of environmental concern, because they have been shown to be mobile, persistent and toxic and some are bioaccumulative<sup>7</sup>. The long term environmental monitoring enables to reveal temporal trends of concentration levels in the marine environment. These data is also necessary to verify if the international measures undertaken to phase-out, reduce losses and emissions of POPs, are effective. There are different ways of performing observation programs but marine mussels, as filtering organisms, have proven to be useful for biomonitoring and assessment of the current state of a site<sup>8</sup>. In our study, we have undertaken the retrospective analysis of “classic” and “novel” persistent organic contaminants in the archived samples of marine mussels (*Mytilus edulis*) collected over the past 22 years within the French National “Mussel Watch” Network (RNO). The aim of this study was to achieve a better

understanding about levels and trends for polychlorinated biphenyls (PCBs), *pp'*-DDT, *pp'*-DDE and *pp'*-DDD ( $\Sigma$ DDT) and brominated diphenyl ethers (BDEs). The comparison between the trends of already banned and novel organohalogen compounds rises new research questions. The analysis in one series and in one laboratory of the archived samples increases the precision of this investigation.

## 2. Methods and Materials

### *Sampling*

The samples analysed were archived freeze-dried samples of blue mussels (*Mytilus edulis*) collected at Villerville in the Seine Estuary during 1981-2002. The location of the sampling site is shown in figure 1. The mussel samples selected for the chemical analysis were collected each year during the same period of late November or early December. This avoids possible differences of contaminant concentrations related to the spawning period and/or physiological states of the mussel. Living mussels were depurated, removed from their shells, homogenized and stored at  $-20^{\circ}\text{C}$  before freeze-drying. Each archived sample consisted of a pooled sample of about 20 mussels of homogeneous size.

### **Extraction and clean-up**

Approximately five grams (dry weight) of the archived mussel sample were spiked with recovery standards (PCB-30, PCB-198 and PCB-209) and extracted twice with dichloromethane using pressurised solvent extraction (ASE, Dionex). Each extraction consisted of five static cycles, two minutes long, at a temperature of  $100^{\circ}\text{C}$  and a pressure of 138 bar. Purification of co-extracted lipids was done by gel permeation chromatography (GPC) on a laboratory prepared column (Bio Beads SX-3 200-400 Mesh). The purified extracts were concentrated and fractionated on a two layer silica/alumina column. Isooctane was added as a keeper solvent to the organic extracts prior the evaporation. Finally, before analysis, the samples were treated with sulphuric acid to remove remaining lipids.

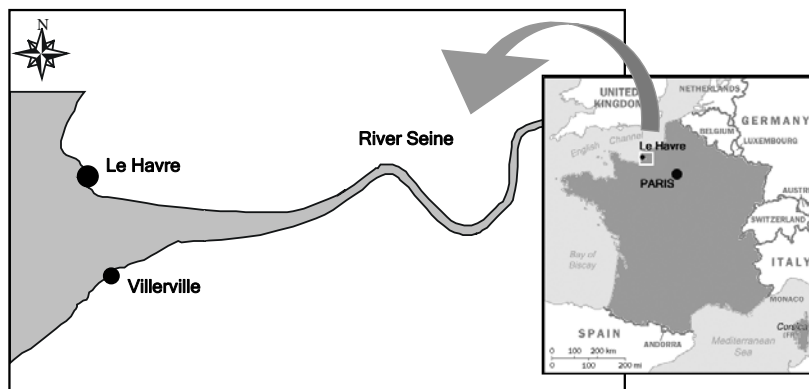


Figure 1. Location of the sampling site Villerville in the Seine Estuary, France.

### Analysis

Polychlorinated biphenyls, *pp'*-DDT, *pp'*-DDE and *pp'*-DDD, lindane and dieldrin were analysed by HRGC using a Varian 3800 with electron capture detector (ECD). Chromatographic separation was achieved by splitless injection (injector 220°C) on a capillary column, 60m × 250µm i.d. × 0.32µm film thickness, using helium as the carrier gas. The oven was programmed to hold at 100°C for 1 minute before increasing the temperature to 180°C at a rate of 7°C/min, followed by a second increase to 280°C at 3°C/min with a final hold for 20 min. The detector had a temperature of 300°C and a nitrogen make up flow of 25ml/min. Identification was achieved by the use of two different columns, DB5 and HT8, to eliminate problems with co-eluting compounds. Quantification was done by the use of external standards. Two calibration curves were used to compensate for the non linear response of the detector, the first in the range of 2-75 pg and the second of 75-250 pg of injected individual congeners.

Polybrominated diphenylethers were analysed by GC/MS using a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett Packard 5973 low resolution mass spectrometer. The utilized technique of analysis was electron capture negative chemical ionisation mode (ECNCI), using methane as the reagent gas. The GC capillary column was a DB1, 15m × 250µm i.d. × 0.10µm film thickness, with a 5m of Siltek-deactivated fused silica guard column. On-column injection was used and the injector was programmed to track the oven temperature. The following temperature program was used: start temperature at 50°C held for 0.5 minutes, followed by an increase to 175°C at 40°C/min, a second increase at 10°C/min to 250°C, and a final increase at 50°C/min to 305°C where it was held for 10 minutes. The flow of the helium carrier gas was 1.2 ml/min. The temperature of the interface was 280°C, the MS source was set to 250°C and the quadrupole to

106°C. Ionisation voltage was 165 eV. Spectra were recorded using selected ion monitoring at  $m/z$  79 and 81 for all BDEs except BDE-209, for which the ions 486 and 488 were recorded. The quantification was made using calibration curves in the range 2-200 pg except for BDE-209, which was calculated using a calibration curve in the range 10-200 pg. BDE-190 was used as the internal standard for all BDEs. The following BDE congeners were determined: BDE-28, BDE-47, BDE-49, BDE-66, BDE-77, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154, BDE-183 and BDE-209. The PCB congeners determined were: PCB-28, PCB-52, PCB-101, PCB-105, PCB-118, PCB-138, PCB-153, PCB-156 and PCB-180. The congeners were only quantified when the  $S/N \geq 5$ . The relative standard deviation (RSD) for the method ( $n=5$ ) was less than 10% for all of the compounds analysed, except for BDE-209, for which it was 30%. Blank samples were prepared by extraction of glass powder. Low levels of BDE-47 and BDE-209 were present in the blanks with concentrations  $\leq 0.06$  pg/ $\mu$ l. Therefore, the samples have not been corrected for blanks.

### 3. Results and Discussion

The overall trend for the levels of selected CBs show a significant decrease during the studied period of time but the differences between individual congeners were large (Figure 2). Between 1981 and 1997, the concentrations of the different CB-congeners decreased with between 40% and 90%. The two dominating congeners CB-153 and CB-138 decreased, respectively, with 41% and 49%. The congener reported to have the lowest concentration, CB-28, showed the largest percentage of

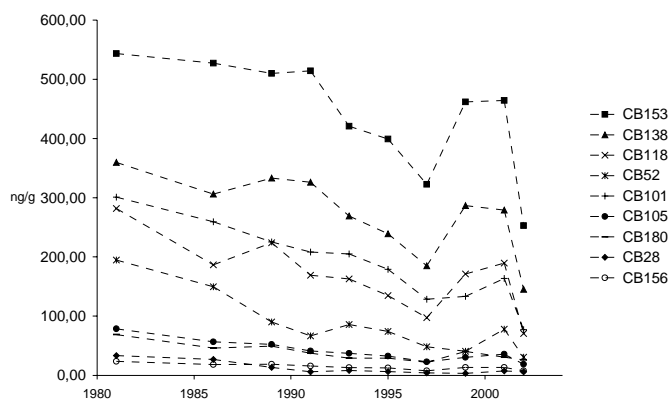


Figure 2. Levels of PCBs (ng/g dry weight) in mussels from the Seine Estuary.

decrease of 90%, i.e. from 33 ng/g dry weight (dw.) in 1981 to 4.1 ng/g dw. in 1997. During 1999 and 2001 a notably increase was observed in the levels of the dominant CB-congeners and the concentrations increased to the same levels as in the early 1990s. In 2002 the levels had dropped to the lowest levels.

Even if the results reveal the differences in the decline of concentrations of lower and higher chlorinated congeners in the mussels from Bay of Seine, it is also shown that the ratio between CB 153 and CB 138, i.e. of higher chlorinated congeners was perfectly constant during the whole period of 22 years (Table 1). These results suggest that the PCB sources were quite same in the Seine estuary during this period of time. However, it is not sure if the lower molecular congeners were degraded and/or witnessed some evaporative losses in this set of archived samples.

Table 1. Concentration of CB-153 and CB-138 ng/g dry weight and the ratio of CB-153 and CB-138.

	1981	1986	1989	1991	1993	1995	1997	1999	2001	2002
CB153	543	527	510	514	421	399	323	462	465	253
CB138	360	306	333	326	269	239	185	287	279	145
153/(153+138)	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6

The levels of organochlorine pesticides  $\Sigma$ DDT (*pp'*-DDT, *pp'*-DDE and *pp'*-DDD) and dieldrin have decreased in mussels from the Seine Estuary between the years 1981 and 2002 with almost 50% loss (Figure 3). During the period 1997-2001 the decrease ceased for  $\Sigma$ DDT, but in 2002 the levels decreased again. For lindane, however, the decrease could not be observed until 1991, but since then the concentration has continued to decrease.

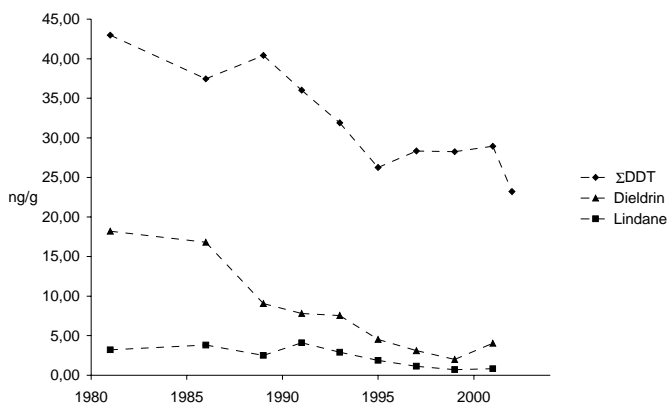


Figure 3. Concentrations (ng/g dry weight) of  $\Sigma$ DDT, dieldrin and lindane in mussels from the Seine Estuary.

The temporal trends of concentrations of the dominating BDE congeners (BDE-47, BDE-99 and BDE-100) in the mussels of Bay of Seine, show that the levels increased rapidly from 1986 (Figure 4). Highest concentrations were found in the samples from 1999 and 2001, that is the same period of a substantial increase in the levels of PCBs. The determined increase of all organohalogens in the mussels at this estuarine station could be related to the important floods of the Seine river during these years. The transport of suspended matter during floods of Seine discharges high amounts of PCBs into estuary. It is also possible that the higher dredging activities contribute to this contamination of the mussels at the mouth of the Seine estuary. Finally, it is also noted that this event has lasted during three years as demonstrated by the drop of the concentrations of almost all studied compounds in 2002.

Low levels of the BDE congeners BDE-28, BDE-66, BDE-154, and BDE-183 were also detected in the samples. The variation of the levels for BDE-28, BDE-66, and BDE-154 were similar with the variation for the congeners displayed in Figure 3, whereas the highest concentration for BDE-183 is observed in the sample

taken in 1991. Low levels were also observed for BDE-209 with the highest concentration in 2001 (0.4 ng/g dw.).

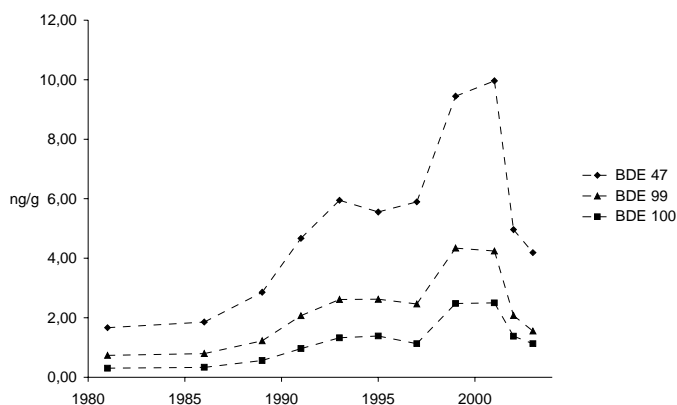


Figure 4. Levels of BDE-47, BDE-99 and BDE-100 (ng/g dry weight) in mussels from the Seine Estuary.

In North America, concentrations of BDEs in environmental sample have been reported to be very high. The levels for some BDE congeners were reported to be in the same range as some PCB congeners<sup>9</sup>. Similar levels for PCB- and BDE congeners have also been observed in sewage sludge in Europe<sup>10</sup>. This is not the case for the mussels of Bay of Seine analysed in this study. The lowest concentration of the most prominent PCB congener, CB-153, observed in this study was 250 ng/g dw. This is 25 times higher than the highest concentration observed for the most prominent BDE congener, BDE-47. The highest concentration for BDE-47 was 10 ng/g dw. and was observed in 2001. In 2001 the concentration for CB-153 was 460 ng/g dw., thus more than 40 times higher than the concentration observed for BDE-47. The sum of the concentrations for the banned  $\Sigma$ DDT isomers (20 ng/g dw.) was also higher than the concentration for the BDE-47 in 2001.

Reported levels of PCBs in fresh water mussels in river Seine upstream of Paris were very low compared to the mussels analysed herein. For CB-153 and CB-138 the concentrations were 28.6 ng/g dw. and 12.8 ng/g dw., respectively<sup>11</sup>. PCBs have also been determined in blue mussels sampled in Greenland 1994-1995 but at very low levels. The mean concentrations for CB-153 and CB-138 were 1.1 ng/g dw. and 0.9 ng/g dw., respectively<sup>12</sup>. The high concentration of PCB in the mussels from Seine estuary are certainly related to significant inputs of PCBs from Paris urban centre.

The temporal trends of concentrations of the different organohalogen compounds studied in the mussels of Bay of Seine reflect relatively well the historical usage of these compounds. The use of DDT in Europe ceased already in the early 70's, with a few exceptions, while PCBs were still used and produced during the late 80's<sup>6</sup>. The global production of PCBs have been estimated to be approximately 1.3 million tonnes, of which more than 70% were tri-, tetra- and penta-chlorinated biphenyls. More than 90% of the produced PCBs were used in the Northern hemisphere, Germany and France were the largest producers and consumers in Europe. The production of PBDEs started in the 1970s but during the 1990s, the use of penta-BDE decreased in Europe due to regulatory measurements<sup>13,14</sup>.

#### **4. Conclusions**

The levels of old halogenated contaminants in blue mussels from the Seine Estuary have decreased during the last 20 years. From 1981 until 2002 the levels of  $\Sigma$ DDT, lindane and dieldrin decreased significantly. The decrease of the PCB levels were relatively slower since the Seine Estuary is highly contaminated with PCBs by constant inputs from Paris - densely populated big urban centre. The levels of novel organohalogen PBDEs have increased since 1981 to 2001. It is only since 2002, the levels of BDEs were found to significantly decrease. A remarkable increase was observed for most of the halogen contaminants during 1999 and 2001. This is most probably related to singular events of an important floods and/or high dredging activities.

#### **5. Acknowledgements**

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