PCDD/F, dioxin-like and markers PCBs in trouts from french aquaculture

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Introduction

Since the introduction of 12 "dioxin-like" polychlorinated biphenyls (PCBs) into the assessment of a tolerable daily intake (TDI) for polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) by the World Health Organization (WHO)[1] in 1998, the analytical determination of non- and mono-ortho PCBs is of increasing interest in the scientific community. The European Commission has already published a regulation that sets maximum limits for dioxins in foodstuffs (Council Regulation 2375/01/EC amending Commission regulation (EC) N° 466/2001 setting maximum levels for certain contaminants in foodstuffs). As an additional feature of the reduction strategy for this group of compounds, the European Commission has planned to include the dioxin-like PCBs in the limit values for food and feeding stuffs starting at the end of 2004. The LABERCA (French National Reference Laboratory for dioxins and dioxin-like PCBs (DL-PCBs)) and the CIPA (French Interprofessional Committee for Aquaculture products) reported levels in French farmed trout according to WHO-TEQ expression and sum of Markers PCBs (m-PCBs). It must be emphasized that this survey only represents a snapshot in time. The results cannot be used to determine the potential contamination of other batches that have not been tested. However, the fish samples were taken from 58 aquaculture sites and 10 fishes were pooled from each site. It is the reason why the results can be interpreted as a good indicative of the contamination levels in farmed trout produced in France.

Materials and Methods

Samples sites

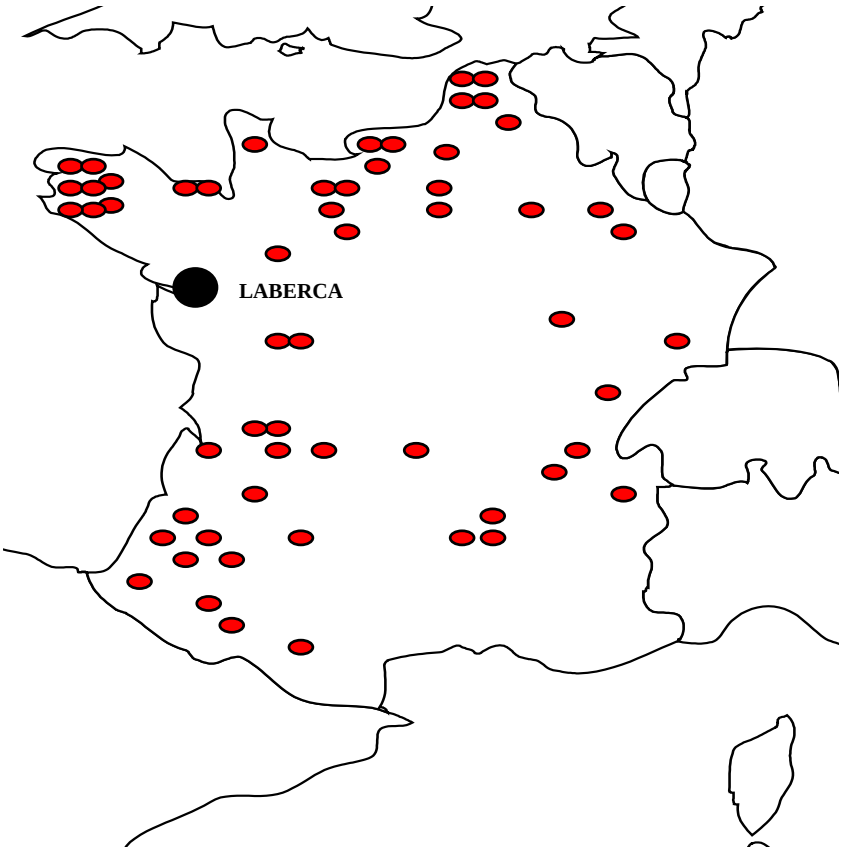
Fish samples were collected in August-November 2001 from 58 different sites in France (Figure 1). These sites were representative of the global French production. The samples were analysed during the period September 2001-February 2002.

Fish sampling and analysis

For each sample, the species was *Oncorhyncus mykiss* and muscle tissue were pooled from 10 individual fishes aged 16-20 months (800-1200 g): 580 farmed trout from 58 locations were collected by inspectors from French veterinary services. The fishes were wrapped in aluminium sheets, packed in polymer bags, frozen, and sent to our laboratory for analysis.

All the organic solvents (Promochem) were Picograde® quality. Silica (Fluka), sodium sulfate and dipotassium oxalate (Merck), acetic acid and sulfuric acid (SDS) were of superior analytical quality. Native and ^{13}C-labeled standards were purchased from CIL. Standard solutions were prepared in toluene for PCDDs/PCDFs and in iso-octane for PCBs and stored in darkness at < 6 °C. 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-step 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.

Figure 1. Map of sample collection sites in France



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/F, $^{13}C_{12}$-PCB #111 for the PCBs), the final sample extract was evaporated under a nitrogen stream to dryness 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. TEQ values were calculated considering the total weight and the fat weight. Contamination profiles, i.e. the relative intensities of each congener, were also studied. 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

Table 1 and Table 2 present mean values for total WHO-TEQ and sum of m-PCBs.

Table 1. Mean values of WHO-TEQ

	In pg WHO-TEQ/g Fresh Weight (FW)		
	TEQ PCDD/PCDF	TEQ DL-PCBs	TOTAL WHO-TEQ
Mean	**0.17**	**0.58**	**0.75**
Minimum	0.02	0.13	0.15
Maximum	0.46	1.51	1.95

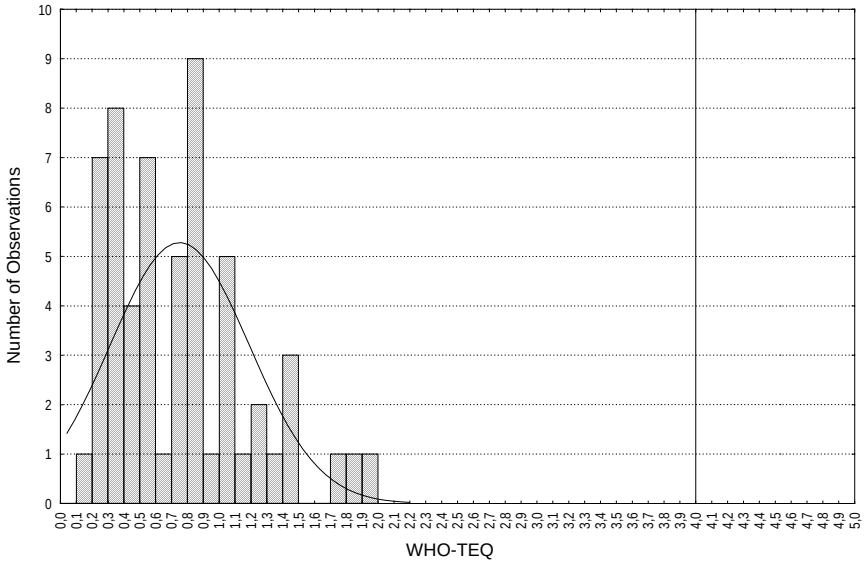
Table 2. Mean values for fat and sum of m-PCBs

	Fat	*Sum of m-PCB*
	in %	*ng /g of fresh weight*
Mean	4.25	6.27
Minimum	1.43	1.30
Maximum	8.78	18.40

The results showed that the incidence of dioxins in French farmed trout were well below the maximum limit set by the pending European legislation. Indeed, current European legislation sets a maximum limit for dioxins of 4 pg WHO-TEQ (PCDD/PCDF)/g wet weight fish. The dioxins levels found in French farmed trouts were on average twenty times under this limit (0,17 pg WHO-TEQ (PCDD/PCDF)/g wet weight fish). The higher level was found at a factor of ten under this limit (0.46 pg WHO-TEQ (PCDD/PCDF)/g wet weight fish).
The DL-PCBs levels were slightly higher (3.5 times higher according to mean value) than the dioxin levels in the same sample. The dioxins and DL-PCBs levels were on average five times under the limit for dioxins and the higher level was only at 1.95 pg WHO-TEQ/g wet weight fish. Figure 2 shows the concentration range frequency.

The levels of the sum of m-PCBs were below the maximum limit fixed at 40 ng/g wet weight fish by the French Food Safety Authority.
At these levels, dioxins and PCBs cannot be considered as a risk for the tolerable levels from the consumption of French farmed trout according to the Tolerable Monthly Intake (TMI) fixed at 70 pg WHO-TEQ/kg.bw by the 57th Joint WHO/FAO Expert Committee on Food Additives and Contaminants. In addition, these levels are comparable with low levels published recently in Ireland[2] and Spain[3].
The aquaculture industry must continue to be vigilant and ensure they use uncontaminated fish feed.

Figure 2. The total WHO-TEQ results; repartition of 58 farmed trouts pools found in FRANCE



Moreover, we tried to demonstrate potential correlations between the three groups of compounds. The correlation between dioxin and "dioxin-like" PCBs has already been observed but the correlation between m-PCBs and DL-PCBs has not been studied.

In Figure 3, we can notice that the correlation between WHO-TEQ (DL-PCBs) and WHO-TEQ (PCDD/F) is rather good ($R^2 = 0.85$; $y = 2.5688x + 0.1347$) and supports the proposals of the DG SANCO Expert Group to set a maximum limit for DL-PCBs at twice the limit for dioxins.

In Figure 4, the correlation between sum of m-PCBs and total WHO-TEQ (PCDD/F-DL-PCBs) is also rather good ($R^2 = 0.88$; $y = 0.0952x + 0.1544$). This result was quite surprising but we had already observed these correlations in our laboratory, although we observed the correlation for other biological matrices. As an example, the correlation between sum of m-PCBs and total WHO-TEQ (PCDD/F-DL-PCBs) for wild fishes consumed in France in 2002 is presented in Figure 5.

Because the correlation between m-PCBs and total WHO-TEQ (PCDD/F-DL-PCBs) is good, it could be an interesting new screening approach to evaluate total WHO-TEQ according to the sum of m-PCBs, determined by GC-ECD or GC-MS.

Figure 3. Correlations between WHO-TEQ (DL-PCBs) and WHO-TEQ (PCDD/F)

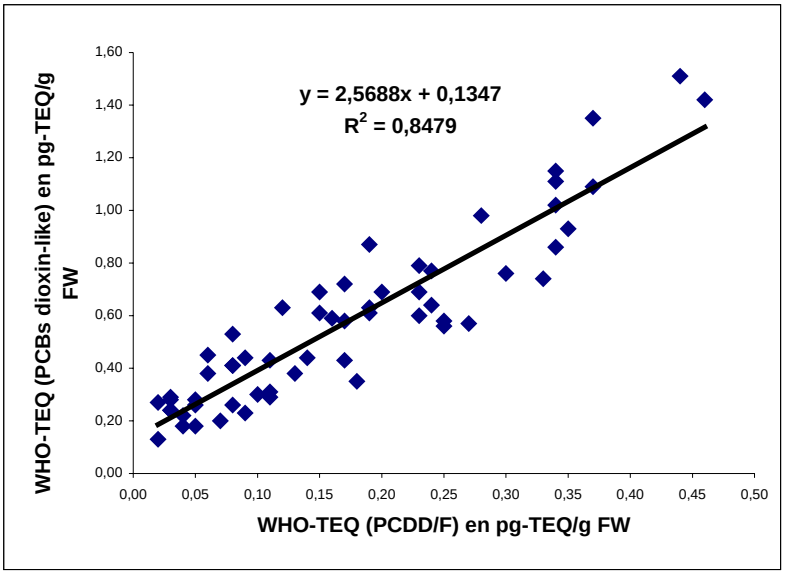


Figure 4. Correlations between Total WHO-TEQ and m-PCBs

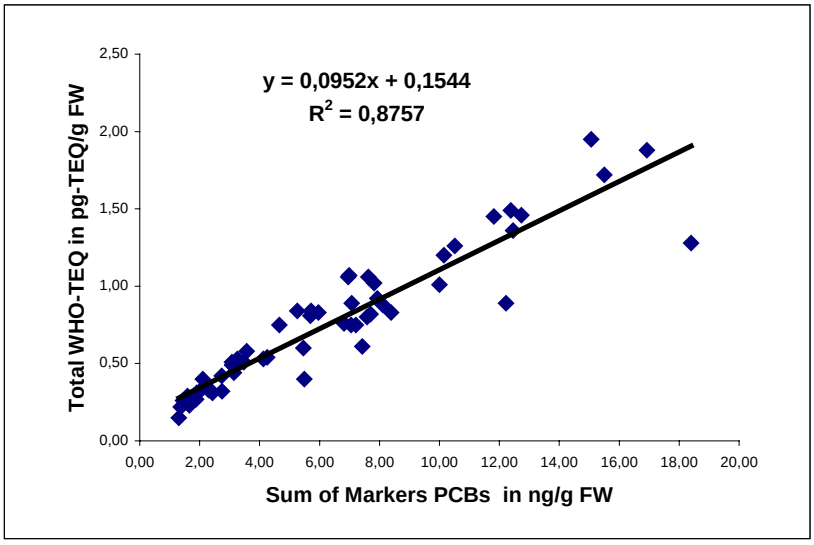
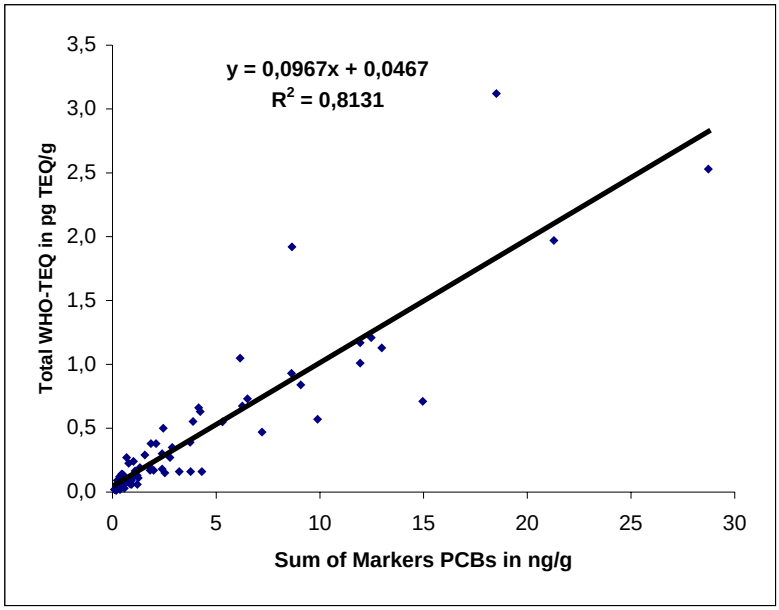


Figure 5. Correlations between Total WHO-TEQ and m-PCBs (n=70) for wild fish from Atlantic Ocean, Mediterranean Sea, Indian Ocean and Pacific Ocean



Conclusion

The total WHO-TEQ results were far below the actual maximum limit which takes into account only PCDD/F. Moreover, we demonstrated a strong correlation between the sum of m-PCBs and the total WHO-TEQ. Indeed, after the fixation of maximum levels for the sum of m-PCBs, their analysis would be an innovative and efficient approach for screening purposes.

Acknowledgements

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