Levels and patterns of PCBs and PCDD/Fs in different tissues of the marine flatfish dab (Limanda limanda) from the English Channel, France.

Catherine MUNSCHY[1], Karine MOISAN[1], Jacek TRONCZYNSKI[1]

[1]IFREMER, Nantes

Introduction

The contamination of marine dabs by selected persistent and toxic organohalogen compounds has been studied in the Eastern part of the English Channel, France. The area receives riverine inputs of diverse chemicals originating from the Seine river basin, a highly industrialized and urbanized zone. The Somme Bay, a less-contaminated zone, has also been investigated. Dab (*Limanda limanda*) is a benthic flat fish commonly found in European coastal waters, and chosen by the International Council for the Exploration of the Sea (ICES) as a sentinel species. The contamination levels of dabs were determined over two sampling periods, in different tissue samples of the pooled fish sorted according to their sex and length. The results presented here are part of a research project carried out to study the chemical contamination and the occurrence of DNA lesions in dab. Primary results have been communicated previously [1,2]. In this paper, the presented results are focused on the contamination of dabs by polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

Methods and Materials

Sampling sites

Fish samples were collected during two sampling cruises in March 2001 and September 2001 on board the OV "Gwen Drez". Samples were collected from different sites located in the French coastal waters of the Eastern English Channel. Four sites were located in the Seine Bay, and two sites were located north in the Somme Bay (Figure 1). The first sampling period (March 2001) corresponds to pre-spawning or spawning of the female dab in this area [3]. The second sampling period corresponds to a non-spawning phase.

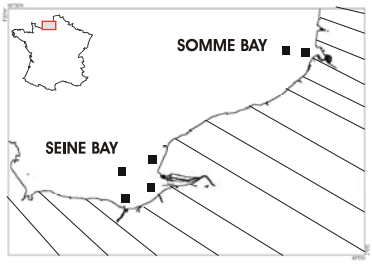

Figure 1: Location of sampling stations

Fish sampling and analysis

Fish were collected using a trawl submerged at a depth of 7-40 metres and then sorted according to sex and length. Two length classes for each study period were considered. The first length class categorised fish between 11-15 cm (March), and 14-18 cm (September), corresponding to fish of one year old to less than two years old (juveniles). The second length class categorised fish between 19-23 cm (March), and 22-26 cm length (September), corresponding to fish of two years old to less than four years old (adults). All fish collected were kept alive onboard until tissue dissection. Samples of muscle, liver, and female gonads for each length class were pooled from fifteen individual fish. These samples were stored at –20°C until further analysis at our laboratory (IFREMER, Nantes, France).

Gravimetric determination of the dry weight of an aliquot of each sample was conducted just before sample treatment. In this paper, the concentrations of contaminants are expressed per dry weight (d.w.) unit of the analysed tissue. Wet weight concentrations are also available. The total amount of extractable lipids was not determined because the quantity of samples were generally not sufficient for lipid analysis. For chemical analysis of organohalogens, five to eight grams d.w. for muscle samples, two to four grams d.w. for liver samples, and nine grams d.w. for gonad samples were taken.

The tissue samples were homogenized, freeze-dried, and spiked before the extraction with a mix of recovery standards (CB-30, CB-198, CB-209, tetrachloronaphtalene -TCN, and sixteen ^{13}C-labelled dioxins and furans). Samples were extracted by dichloromethane (DCM) using Accelerated Solvent Extraction (ASE, Dionex Corp., CA). Extracts were cleaned on a Gel Permeation Chromatography (GPC) column eluted with DCM, fractionated on a silica and alumina column, treated with concentrated sulphuric acid and further fractionated using a two-dimensional High Performance Liquid Chromatography (HPLC) system with two columns coupled in series. The first column, a nitrophenylpropylsilica column (Nucleosil, 5-µm particles, 250 x 4.6 mm, Interchim, France), was used in combination with a 2-(1-

pyrenyl)ethyldimethylsilylated silica (PYE) column (Cosmosil, 5-µm particles, 150 x 4.6mm, Promochem, France) as described in [4]. The analytical method developed enables the separation of the same organic extract in 3 fractions: the first one contained the PCBs (non-ortho PCBs excluded), the second one contained the non-ortho-PCBs, and the third one, eluted in backflush mode, contained the PCDD/Fs.

The analysis of PCBs within dab tissue employed the technique of High Resolution Gas Chromatography (HRGC) (GC3800, Varian, CA, USA) equipped with an Electron Capture Detector (ECD), using two columns of different polarities: a DB5 (5% phenyl-methylpolysiloxane) column (60m x 0.25 mm, film thickness 0.25µm), and a HT8 (8% phenyl-polysiloxane-carborane) column (50m x 0.22 mm, film thickness 0.25µm). All PCBs were quantified on both columns in order to avoid misinterpreting quantitation because of co-eluting congeners, and the reported concentrations were chosen on the appropriate column for each congener. Concentrations were calculated by external multi-level linear regression calibration, in the linear range of the response of the detector. The standards added before the extraction were used as recovery surrogates, but concentrations were not corrected by the recovery of the surrogate standards. Thirty congeners with 3 to 8 chlorine atoms were analysed including the dioxin-like mono-ortho (IUPAC N°105, 118, 123, 156, 167, 189) and non-ortho congeners (IUPAC N°77, 126, 169) from the World Health Organisation (WHO) priority list.

Analyses of PCDD/Fs were performed by HRGC-HRMS (High Resolution Mass Spectrometry) with an AutoSpec Ultima (Micromass, Manchester, UK) operated in Electronic Impact (EI) ionisation mode at a resolution of 10 000 in the Selected Ion Monitoring (SIM) mode, and equipped with a Hewlett-Packard (Palo Alto, CA, USA) 6890 GC and a RTX-5 column (Restek), 40 m x 0.18 mm i.d., 0.2 µm film thickness. Seven PCDDs and nine PCDFs were quantified for individual components by isotopic dilution based on the US EPA 1613 method. Mean recoveries +/- standard deviation (n=31) of individual labelled congeners were between 77 +/- 7 % and 109 +/- 7 %. Congener 2,3,4,6,7,8-HxCDF could not be quantified because of important quantitative loss during the analytical procedure. Final values were corrected by blank substraction.

Quality Assurance / Quality Control (QA/QC) procedures (blank determinations, analysis of replicates and certified materials) were included within every batch of six to eight samples. The laboratory is also routinely participating in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) intercomparison exercises.

Results and Discussion

PCDD/F congener concentration levels and patterns in dabs

Concentrations of total PCDD/Fs (the sum of seven PCDDs and nine PCDFs congeners) in adult female dab muscle were between 14.2 pg g^{-1} d.w. and 7.3 pg g^{-1} d.w in March, and between 6.0 pg g^{-1} d.w. and 2.7 pg g^{-1} d.w. in September, for both sampling sites. The highest concentration levels were recorded at sites located in the Seine Bay, whereas the samples collected at sites in the Somme Bay exhibited less contamination. Concentrations in liver of the same fish were between 358.3 pg g^{-1} d.w. (Seine Bay) and 89.4 pg g^{-1} d.w. (Somme Bay) in March, and between 147.9 pg g^{-1} d.w. (Seine Bay) and 61.5 pg g^{-1} d.w. (Somme Bay) in September. These differences in the contamination levels between both sampling areas are related to the higher inputs of various contaminants from industrial and urban sources into the Seine river estuary than into the Somme river estuary [5]. These results suggest also that, despite not being considered as a sedentary fish, the adult female dab cohorts collected in each sampling site do not mix to a significant extent. The concentrations of PCDD/Fs in liver were between 11 and 40 times higher than those in muscles and are related to the higher lipid content in the liver and the higher affinity of hydrophobic compounds for lipid-rich tissues [6]. The concentration of PCDD/Fs determined in a sample of female gonads from the Seine Bay in March was 14.8 pg g^{-1} d.w., i.e. 11 times lower than in liver and 1.6 times higher than in muscle.

The PCDD/F concentrations in dabs from the Seine Bay were higher than those reported for fish collected in the Adriatic Sea [7], the latter fish exhibited higher concentrations for PCBs. Among the French coastal waters, the Seine Bay has been reported to be the most highly contaminated by PCDD/Fs [8]. Levels in our samples are in the range of those reported in marine fish from the Netherlands, although slightly lower for PCDDs [9].

For all tissue samples, the contamination patterns were dominated by PCDFs, representing more than 80% of the sum of PCDDs and PCDFs. The contamination pattern (mean pattern for n=12 samples) determined in liver samples for individual isomers expressed as a percentage of the sum of PCDD/Fs is shown in Figure 2 for female adult dabs from the studied area. Similar patterns were observed in liver, muscle and female gonads, and for both sampling areas. Furthermore, no difference was recorded for the congener distribution between males and females. The similarity of PCDD/Fs composition in all of our samples suggests that in the studied area, the dominant source emits a fairly homogeneous suite of these compounds. The similarity in the contamination patterns of fish of different sexes and ages has been observed by other authors [10], and has been attributed to the fact

that the range of PCDD/Fs octanol-water partition coefficients (Kow) is narrow (log Kow is 6.5 to 9.3 [11]), and not in the optimum of bioaccumulation to induce detectable differences in the contamination patterns of fish. Among PCDF congeners, 2,3,7,8-TCDF was the most abundant, followed by 2,3,4,7,8-PeCDF, the latter compound originating from technical PCBs and combustion processes [12]. Whereas, PCDFs, and 2,3,7,8-TCDF in particular, originate primarily from the combustion of PVC and from PCB formulation [13,10]. The predominant PCDD isomers are 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and 2,3,7,8-TCDD.

The patterns determined within this study are similar to those already reported for marine and estuarine fish [9,10]. However, the OCDD isomer has sometimes been found in a higher proportion in fish and in sediment samples from different areas [14,6]. The OCDD isomer is produced in high proportion in sewage sludges [13], and in combustion byproducts of fuel oil mixtures [15]. Our results suggest that fish from the studied area are exposed to PCDD/Fs originating mainly from combustion processes. However, the pattern observed in fish tissues is not only the result of exposition but depends also on different factors such as uptake efficiency, metabolism, and excretion. The uptake efficiency of PCDD/Fs in fish is reported to be less efficient for heptachlorinated congeners than for lower chlorinated compounds, whereas metabolism of PCDD/Fs in fish has been reported as a mechanism that is highly species-dependant [16,17].

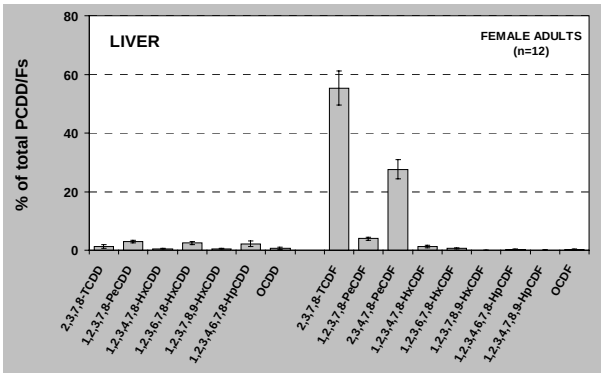

Figure 2: Normalised pattern of individual PCDD/F congeners in liver samples (mean pattern for n=12 samples) in female adult dabs collected during both sampling cruises in the Seine Bay and the Somme Bay.

PCB congener concentration levels and patterns

The concentrations of PCBs determined in samples collected from the Seine Bay were also higher than the concentrations in samples from the Somme Bay. The highest PCB concentrations were recorded in those fish that exhibited the highest concentrations of PCDD/Fs. Summed concentrations of the ICES seven congeners in the muscle of female adult dabs collected from the Seine Bay sampling sites were between 52 ng g^{-1} d.w. and 90 ng g^{-1} d.w. in March, and between 23 ng g^{-1} d.w. and 41 ng g^{-1} d.w. in September. In samples from the Somme Bay, the concentrations were between 27 ng g^{-1} d.w. and 31 ng g^{-1} d.w. in March and between 12 ng g^{-1} d.w. and 13 ng g^{-1} d.w. in September. These congeners represented more than 70% of the total amount of PCBs quantified in our samples. The concentrations in liver of the same samples were in the 792-1665 ng g^{-1} d.w. (March) and 759-1479 ng g^{-1} d.w. (September) ranges in fish from the Seine Bay, and in the 170-439 ng g^{-1} d.w. (March) and 210-219 ng g^{-1} d.w. (September) ranges in fish from the Somme Bay. The concentrations in the gonads of the same fish were determined in a limited number of samples. They were approximately two times higher than in muscle, and three to eight times lower than in liver. For the March results, the concentrations were 52 ng g^{-1} d.w. in the Somme Bay, and 99 ng g^{-1} d.w. in the Seine Bay. The concentration levels in the gonads of female dabs from this zone have been reported to be at a highest during March to April, and to show a drastic decrease in June after the spawning [18].

The concentration levels of the mono-ortho (CB-105, CB-118, CB-156) and di-ortho (CB-153) congeners determined in both muscle and liver of dabs from the Seine Bay were higher than those reported in the literature for dabs from the German Bight and the Wadden Sea [19]. Contrary to what was observed for PCDD/Fs, the concentrations of the sum of the seven ICES congeners in dabs from the Seine Bay were lower than those found in different fish species from the Adriatic Sea [7].

The contamination patterns in liver were dominated by hexa-, penta-, and hepta-chloro congeners, and were similar to the patterns determined in muscle. In Figure 3, the concentrations of individual congeners were reported as normalized to the most abundant congener IUPAC N°153. The PCB pattern observed in fish reflects their exposition to an ambient composition of contaminants, but also depends on elimination and metabolic transformation, which is markedly species-dependent in fish [20,21,22].

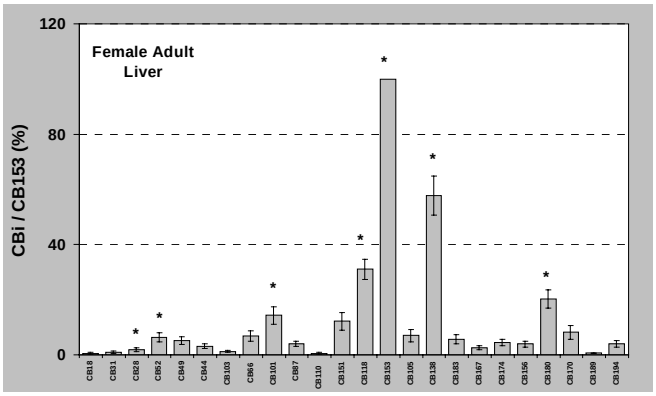

Figure 3: Congener-specific mean pattern (n=13) of PCBs normalized to CB-153 in adult female dab livers from the studied area. Asteriks are for ICES congeners. Error bars are for standard deviations.

Planar PCBs

Non-ortho congeners CB-77, CB-126, and CB-169 were found to represent, in dab muscle and liver respectively, between 0.14 % and 0.34 %, and between 0.14 % and 0.43 % of the total PCB concentrations. The concentration levels were between 44 pg g^{-1} d.w. and 196 pg g^{-1} d.w. in muscle, and between 846 pg g^{-1} d.w. and 4622 pg g^{-1} d.w. in liver, for all sampling sites and both sampling periods. The concentration levels of these three congeners in liver samples from the Seine Bay are higher than those reported in the liver of dabs from the German Bight and the Wadden Sea [19], whereas the concentrations determined in the muscle are in the range of those reported in dabs from the Wadden Sea. However, the concentrations of planar PCBs reported for different marine fish species collected from the Netherlands and other areas in the world exhibit a very broad range, depending on the species and the site. Among the three planar congeners, CB-77 was the most abundant, accounting for, on average, 74 % of the sum of CB-77, CB-126 and CB-169. These relative abundances are usually observed in marine and freshwater fish [14,19], and are similar to the composition of the technical mixture [19], thus suggesting that metabolic degradation of these congeners in dabs is low.

2,3,7,8-TCDD Toxic Equivalents

The 2,3,7,8-TCDD Toxic Equivalents (TEQs) of PCDD/Fs and PCBs in the muscle of adult female dabs calculated with the Toxic Equivalency Factors (TEFs) for fish [23] were 0.655 pg TEQ g^{-1} wet weight (w.w.) and 0.473 pg TEQ g^{-1} w.w. in the Seine Bay and the Somme Bay, respectively. Among the different congeners, 2,3,4,7,8-PeCDF contributed the most to the total toxicity (>50%), followed by

1,2,3,7,8-PeCDD (12-16%), 2,3,7,8-TCDF (8-13%) and PCB congener IUPAC N°126 (6-8%). Using the TEFs reported by the WHO, the corresponding values were about 3 times higher, and the non-ortho congener of IUPAC N°126 contributed the most to the toxicity (48-50%). The TEQs calculated in our samples were lower than the recommended limit of 3 pg TEQ g^{-1} w.w. set by the European Community for sea products intended for human consumption [24]. However, this limit does not take into account the contribution of PCBs. As the estimation of TEQ is highly dependent upon the TEF used for the calculation, and that the TEF concept includes some uncertainty, we strongly recommend the communication of data on an individual-congener concentration basis, especially in studies of environmental concern.

Acknowledgements

This work was supported by the "Agence de l'Eau Seine Normandie" in France.
We would like to thank the crew of the French Oceanographic Vessel "Gwen Drez" for valuable help during the sampling cruises. We are highly thankful to Professor E. De Pauw, G. Eppe, and their team from the CART (Centre d'Analyse des Résidus en Traces) at the University of Liege, Belgium, for helping in GC-HRMS analysis.

References

1. Munschy C., Moisan K., Tronczynski J., (2003). Organohalogen Compounds, 62, 157-160.
2. Akcha F., Vincent-Hubert F., Leszkowicz A., (2003). Mutation Research, 534, 21-32.
3. Tassel M. (1988). PhD thesis, Université de Lille-Flandres-Artois, 236p.
4. Bandh C., Ishaq R., Broman D., Näf C., Rönquist-Nii Y., Zebühr Y., (1996). Environ. Sci. Technol., 30, 214-219.
5. Johansson I., Guiot N., Moisan K., Munschy C., Tronczynski J., (2004). Submitted to Organohalogen Compounds.
6. Wu W.Z., Schramm K.W., Xu Y., Kettrup A., (2001). Chemosphere, 43, 633-641.
7. Bayarri S., Baldassarri L.T., Iacovella N., Ferrara F., Domenico A., (2001). Chemosphere 43, 601-610.
8. Abarnou A., Fraisse D., (2002). Organohalogen Compounds, 56, 469-472.
9. Liem A.K.D., Theelen R.M.C., (1997). National Institute of Public Health and the Environment, Bilthoven, The Netherlands. Thesis, 373 p.

10. Fernandez M.P., Ikonomou M.G., Courtenay S.C., Wirgin I.I., (2004). Environ. Sci. Technol., 38, 976-983
11. Paasivirta J., Sinkkonen S., Mikkelson P , Rantio T., Wania F., (1999). Chemosphere, 39, 5, 811-832.
12. Tanabe S., Watanabe M., Minh T.B., Kunisue T., Nakanishi S., Ono H., Tanaka H., (2004). Environ. Sci. Technol., 38, 403-413.
13. Baker J.I., Hites R.A., (2000). Environ. Sci. Technol., 34, 14, 2879-2886.
14. Loganathan B.G., Kannan K., Watanabe I., Kawano M., Irvine K., Kumar S., Sikka H.C., (1995). Environ. Sci. Technol., 29, 1832-1838.
15. Hellou J., Payne J.F., (1993). Mar. Environ. Res., 36, 117-128.
16. Van den Berg, M., De Joong J., Poiger H., Olson J.R., (1994). Crit. Rev. Toxicol., 24, 1-74.
17. Knutzen J., Bjerkeng B., Naes K., Schlabach M., (2003). Chemosphere, 52, 745-760.
18. Loizeau V., Abarnou A., (1994). Mar. Environ. Res., 38, 77-91.
19. de Boer J., Stronck C.J.N., Traag W.A., van der Meer J., (1993). Chemosphere, 26, 10, 1823-1842.
20. Boon J.P., Eijgenraam F., Everaarts J.M., (1989). Mar. Environ. Res., 27, 159-176.
21. Goerke H., Weber K., (2001). Mar. Environ. Res., 51, 131-149.
22. Jacobs M.N., Covaci A., Schepens P., (2002). Environ. Sci. Technol., 36, 2797-2805.
23. Van den Berg, M.V., Birnbaum, L., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk,D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., (1998). Environ. Health Persp. 106, 12, 775-792.
24. 2002/201/CE, (2002). Journal officiel des communautés européennes, C (2002) 836.