ASSESSMENT OF THE EXPOSURE OF SOLE (*SOLEA SOLEA L.*) TO POLYCHLORINATED BIPHENYLS (PCBS) *VIA* SEDIMENT

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Introduction

In the coastal marine environment, the sediment compartment may act as a burial sink and also as a source of persistent hydrophobic organic contaminants (HOCs) to water and benthic organisms. HOCs could be released from sediment bed to overlying water by various physico-chemical processes, such as diffusive exchanges, bioturbation or particles resuspension, and hence become bioavailable to organisms. This study aimed at a better understanding of the transfer of persistent organic pollutants such as polychlorinated biphenyls (PCBs) from marine sediment to benthic fish. Common sole (*Solea solea L.*) was chosen as the target species for this study, in view of its benthic habitat and of its potential exposure to sediment-associated contaminants.

Under laboratory-controlled conditions, soles were exposed to natural sediments collected in an area contaminated by PCBs, the Seine Estuary, France. In parallel, low-density polyethylene (LDPE) strips were used to assess contaminant exchanges between sediment and water during the experiment. Passive sampling with LDPE strips has been previously successfully used to assess freely dissolved concentrations of various HOCs in water bodies [1,2,3]. PCB levels and congener patterns in fish were compared with those obtained in total sediment, water and LDPE strips.

Materials and methods

LDPE strips preparation: Single layered strips (50 cm long) were prepared from low-density polyethylene (LDPE) lay-flat tubing (2.5 cm wide) containing no additives (Brentwood plastics, MO, USA). Strips were pre-extracted twice with n-hexane and spiked with five Performance Reference Compounds (PRCs) covering a log K_{OW} range of 4.84 (CB 10) to 7.3 (CB 204)[4]. Strips were kept in a freezer (-20^{o}C) until use.

Exposure of soles to natural contaminated sediments: Surface sediments were collected in the Seine estuary, an area highly contaminated by PCBs and one of the main nursery zones for the common sole (*Solea solea* L.). In order to reach chemical equilibrium, sediments (6-7 L) were placed in aquariums (120 L) with regularly renewed sea water during one month. Ten juvenile soles (0-age) obtained from a commercial hatchery (Solea BV, Ijmuiden, Netherlands) were then placed in each aquarium (one to four aquariums were used at each exposure time). Control experiment was conducted with soles exposed to commercial sand free from PCB contamination (baked in the oven at 450°C). Both exposed and control fish were fed the same commercial food (Boschetto-Frozen fish food). In order to follow the exchanges between sediment and water, LDPE strips were exposed to mid water in the same aquariums. Fish and LDPE strips were collected for chemical analysis at selected exposure times (14, 30, 65 and 91 days). Experiments were performed at a constant temperature (20°C).

Preparation of samples and analytical procedure: All fish were anaesthetized, measured, weighed and brought to the laboratory for further dissection in clean conditions. Muscle and skin tissues were collected from individual fish and weighted. Samples were made from pooled individuals from the same aquarium (n = 7 to 10). Sediment and fish samples preparation was conducted according to Hong *et al*.[5]. After exposure, LDPE strips were washed with milli-ro water and extracted twice by soaking overnight in cyclohexane spiked with recovery standards. The clean-up and fractionation of LDPE extracts were the same as the one used for sediment and fish tissue extracts.

PCBs were analysed by GC/ECD according to the procedure described earlier[6]. Twenty-five PCB congeners (IUPAC numbers: CB 18, 28, 31, 44, 49, 52, 60, 66, 87, 101, 103, 105, 110, 118, 138, 151, 153, 156, 167, 170, 174, 180, 183, 189, 194) were investigated. Internal QA/QC procedures, including laboratory and field blanks, analyses of replicate samples and use of recovery standards added to each sample before extraction, were followed for each batch of samples.

Determination of dissolved PCB concentrations in water: Based on the exposure of LDPE strips to the water of the aquarium, time-weighted averaged (TWA) concentrations of PCB in water were calculated according to the following equation[7]:

$$C_W = \frac{C_{LDPE}}{K_{LDPE\_W}\left(1 - \exp\left(-\frac{R_S t}{M_{LDPE} K_{LDPE\_W}}\right)\right)} \qquad \textit{(Equation 1)}$$

where C_W is the TWA concentration of dissolved PCB in water (ng/L), C_{LDPE} the concentration of PCB the LDPE strip (ng/kg$_{PE}$), M_{LDPE} the mass of the LDPE strip (kg), K_{LDPE_W} the LDPE-water partition coefficient of PCB[8] (L/kg), R_S the sampling rate of PCB (L/day) and t the exposure duration (day). Rs were determined from the dissipation of all PRCs from the LDPE strips according to the method proposed by Booij and Smedes[9].

Non linear and linear Regression: **N**on-linear and linear regressions were performed in the Origin System 7.5 SR2 version 7 (OriginLab Corporation, MA, USA). All statistical analyses were performed using Statistica software for Windows version 7.1 (StatSoftInc.) with a significance level of $p < 0.05$.

Results and discussion

Characterisation of the sediments used for fish exposure

CB 153 was the main congener in the natural sediments used, with a concentration of 12.5 ng/g dry weight (dw). The other main congeners, i.e., CB 101, 110, 118, 138 and CB 180, presented concentrations higher than 5 ng/g dw. Analysis of the sand used for control experiments revealed no traces of PCBs, with a limit of quantification of 5 pg/g dw.

Exchanges of contaminants between sediment and water in the aquarium

For fish viability reasons, the water of each aquarium was renewed twice a month (50 to 75% each time), hence possibly leading to the removal of PCBs from the exposure media. Thus, water concentrations calculated below were only indicative values and must be considered cautiously.

After three months of exposure, the analysis of LDPE strips exposed in the aquariums containing natural sediments revealed PCB freely dissolved concentrations ranging from 2 pg/L for CB 194 to 151 pg/L for CB 52, and a total PCB concentration (sum of 25 congeners) of around 1070 pg/L. Total PCB concentration in control aquariums was 70 pg/L, with a maximum concentration of 15 pg/L for CB 28. These levels were negligible compared to the ones determined in aquarium containing natural sediments. This demonstrates that PCBs were released from sediment to overlying water, and shows that the natural sediment constituted the main source of contamination of the water in the aquarium, and thus of the fish.

Contamination levels of soles exposed to natural contaminated sediments

This study was designed to assess the bioaccumulation of contaminants in two fish tissues: muscle and skin. Muscle constitutes the main storage compartment, in mass, for the fish. Bioaccumulation in the skin was also determined as skin may be considered as a potential uptake route of contaminants for the fish and also as a potential lipid storage compartment in sole. The results are presented here for twelve PCB congeners including the seven ICES indicator PCBs (CB 28, CB 52, CB 101, CB 118, CB 138, CB 153, CB 180), three mono-ortho substituted PCB (CB 105, CB 156, CB 189), CB 31 and CB 110. Figure 1 presents the accumulation of these congeners in muscle of soles exposed to sediments and of control soles at each exposure time. Over the whole period of exposure, control sole muscle presented a low contamination level of 15 ± 2.5 ng/g dw (sum of 12 congeners ± standard deviation over the whole period of experiment). A regular increase of the contamination levels in the muscle of sole exposed to natural sediment was observed with time: after three months of exposure, the contamination level was around eight times higher than the one determined in control fish. The same increase was observed when lipid-normalized concentrations were considered.

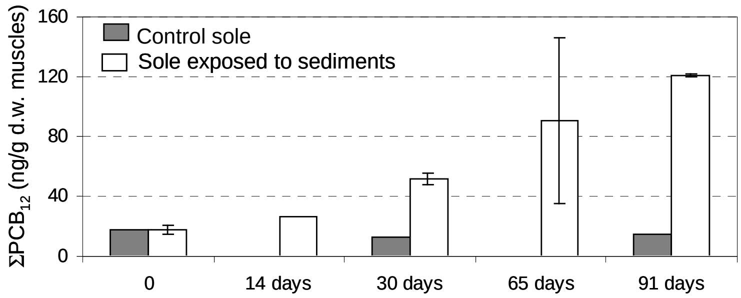

Figure 1 : Accumulation of PCBs in muscle of control soles and soles exposed to sediments at different exposure times. Sum of 12 congeners ± standard deviation (2 replicates) : CB 31, CB 28, CB 52, CB 101, CB 110, CB 118, CB 153, CB 105, CB 138, CB 156, CB 180 and CB 189

Contaminant accumulation in sole muscle obeyed a linear kinetic law (data not showed). Accumulation rates ranged from 1 pg/g muscle/day for CB 189 to 288 pg/g muscle/day for CB 153.
Similar results were obtained for the bioaccumulation of PCB congeners in skin: PCB levels in the skin of fish exposed to sediment were significantly higher than those of control fish and a regular increase of the contamination level was observed with time for lipid normalized concentrations in skin. Lipid-normalized concentrations in muscle and skin were quite similar. After three months of exposure, the contamination levels (sum of 12 PCBs) were around eight times higher in exposed fish than in control fish. Whichever tissue was considered, an increase of PCB contamination levels was thus observed during the exposure of soles to sediments, demonstrating the exposure of sole to PCBs via sediment.

Contamination pattern of soles exposed to natural contaminated sediments
The contamination patterns in the muscle of control soles (mean value over the experiment total duration) and of soles exposed to sediments after various exposure times are presented on figure 2. A significant increase ($p<0.05$) of CB 101, CB 110 and CB 118 relative percentages (mean over the total duration) was observed in exposed fish compared to control fish. These congeners are among the most abundant in the analysed natural sediment, suggesting that fish exposure to sediment led to a significant modification of the contamination pattern in muscle compared to the control fish.

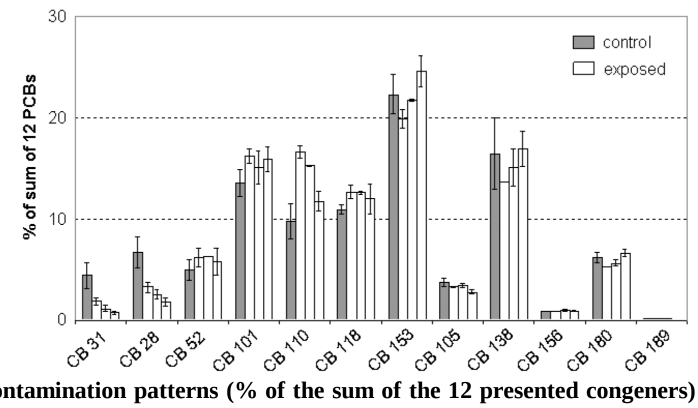

Figure 2: Contamination patterns (% of the sum of the 12 presented congeners) in the muscles of control fish (mean +/- sd at 0, 30 and 91 days of exposure) and of fish exposed to sediments for 30, 65 and 91 days

On the other hand, a significant ($p<0.05$) decrease of the proportion of low chlorinated congeners (CB 31 and CB 28) with time was observed in the muscle of soles exposed to natural sediments. This decrease was also observed in control fish (data not shown). This modification in contamination patterns observed in both exposed and control soles could indicate a qualitative change in the exposure to PCBs of fish before and during the experimentation (change of food, environmental conditions…).
No significant difference was observed between the contamination patterns in muscle and skin for fish exposed

to sediments. At the time scale considered in this study, the accumulation in both tissues did not seem to obey different dynamics.

Comparison of PCB uptake by LDPE strips and sole

Figure 3 presents the contamination patterns of the muscle of soles and of the LDPE strips exposed to mid-water after three months of exposure to natural sediment. Significant differences were observed for several congeners. The accumulation in muscle did not seem to be only due to passive processes such as passive diffusion of dissolved contaminants to a hydrophobic compartment (illustrated here by the LDPE strips). Other processes such as direct ingestion of particles, possible metabolisation or elimination of certain PCB congeners have to be taken into account[7, 10, 11].

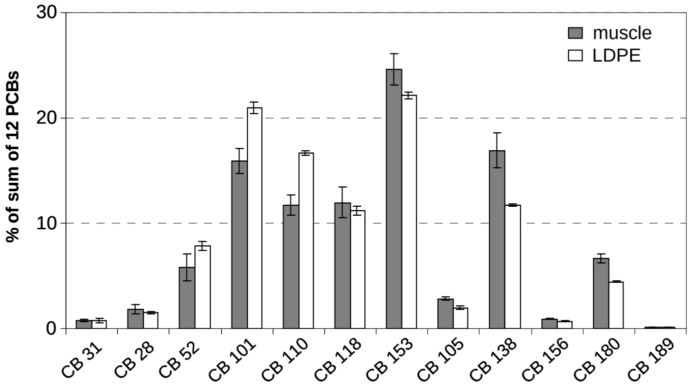

Figure 19: Contamination patterns (% of sum of the 12 presented congeners) of sole muscles (mean +/- standard deviation on two pools) and LDPE strips exposed to mid-water of the aquarium (mean +/- standard deviation on four aquariums) after an exposure time period of 91 days to sediment

Acknowledgements:

Funding of this work was from Onema (Office National de l'Eau et des Milieux Aquatiques), the French national agency for water and aquatic environments. This study was conducted under the approval of the Animal Care Committee of France under the official licence of M.L. Bégout (17-010).

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