Accumulation of PCBs by Atlantic Cod

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ACCUMULATION OF POLYCHLORINATED BIPHENYLS FROM CONTAMINATED SEDIMENT BY ATLANTIC COD (*GADUS MORHUA*) – DIRECT ACCUMULATION FROM RESUSPENDED SEDIMENT AND DIETARY ACCUMULATION VIA THE POLYCHAETE *NEREIS VIRENS*

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Abstract

Bioaccumulation of sediment associated polychlorinated biphenyls (PCBs) was examined in Atlantic cod (*Gadus morhua*) through (1.) direct diffusion from the sediment (via the water phase), and (2.) through the food chain (dietary exposure). To facilitate direct accumulation from the sediment, the sediment was continuously resuspended. To study the dietary bioaccumulation of PCBs, cod were fed benthic polychaetes (*Nereis virens*) previously exposed to test sediments, i.e. "naturally" polluted sediments from the inner Oslofjord (Norway). Both exposure experiments had duration of 129 days. Furthermore, the role of sediments as source of PCBs accumulated in Oslofjord cod was elucidated, using results from environmental monitoring as a reference. Generally, the results suggest that the contaminated sediments of the inner Oslofjord are an important source of legacy PCBs for accumulation in resident cod, although additional contributions also may be important. Crude estimates of assimilation efficiency of ingested PCBs (through diet) was found to be 30-50%; highest for the lower chlorinated congeners (PCB-28 and -52). Challenges for applying Trophic Magnification Factors (TMF) for determining biomagnification in laboratory experiments, in terms of preventive environmental safety, are indicated. The results provide useful information for parameterization of models describing the behaviour of hydrophobic persistent contaminants in the foodweb of the Oslofjord and elsewhere.

Key Words: Bioaccumulation, PCB, *Gadus morhua*, sediment, *Nereis virens*

Introduction

Polychlorinated biphenyls (PCBs) and bioaccumulation processes

The identification of polychlorinated biphenyls (PCBs) in samples of biota by Søren Jensen in the 1960s [1] initiated extensive investigation on their abundance in the environment, and their distribution throughout the biosphere is now well documented [e.g. 2-4]. The banning of PCBs in several countries was to follow in the 1970s and caused the global PCB production to decline. One important international agreement in this regard is the Stockholm Convention on persistent Organic Pollutants (POPs), which is a global treaty to protect human health and the environment from hazardous substances by restricting and ultimately eliminating their use, trade, release and storage. Worldwide, significant quantities of PCBs are however still in present in old infrastructure and equipment. Some PCBs are shown to have various toxic effects (Reviewed by Safe [5]), including immunosuppressive and endocrine disrupting effects, as well as impairment of reproduction.

The environmental fate of contaminants, such as PCBs, is an important ecotoxicological aspect, and bioaccumulation is a fundamental phenomenon in this regard. For a chemical to bioaccumulate, it must be available (bioavailable), and once bioaccumulated, a contaminant may (dependent on its physico-chemical properties) be further subject to biomagnification (the chemical concentration in an organism exceeds that in its diet after dietary absorption [6]). In aquatic organisms, bioaccumulation is the process that causes an increased chemical concentration in the organism compared to that in its ambient environment, water and/or sediment [7]. Recently a group of experts has suggested the following definition of a

bioaccumulative substance in a regulatory context: a substance is considered bioaccumulative if it biomagnifies in food chains [8].

It is well known that because of their persistence and lipophilicity, PCBs have the potential to bioaccumulate and biomagnify in food chains . The highest concentrations of these compounds are found in top predators like seabirds and marine mammals [e.g. 3, 4].

Other persistent organic pollutants (POPs) share similar physicochemical properties as some of the PCBs (for instance polybrominated diphenyl ethers, PBDEs and hexachlorocyclododecane, HBCD; [9, 10]). Therefore, results obtained from bioaccumulation studies where PCBs are employed as the model compounds may to some extent serve as valuable information with regard to POP bioaccumulation processes, in general.

Aquatic organisms take up PCBs and other lipophilic substances through the ingestion of food and directly from water through passive diffusion at the body surface, mainly via the respiratory surfaces. Several models have been introduced to describe these processes (reviewed by Mackay and Fraser [7]). Which of these routes that are the most important for bioaccumulation may vary between organisms with different modes of living, and have been the subject of much discussion (See below; [e.g. 7, 11, 12]). Bioaccumulation is the net result of uptake and elimination (the latter through metabolic transformation, reproductive losses, fecal egestion, or diffusive fluxes [13, 14]). The capability of metabolic transformation of PCBs by fish is however limited, and fecal elimination has been shown as a no important loss mechanism [13]. Mechanistic mass balance models may be built where the different uptake and elimination processes are quantified. These models have the advantage that they may take into account effects of phenomena like

compound specific biotransformation rates and ‘growth dilution’ [7]. They are, however, in need of sound parameterization.

Environmental monitoring

The Coordinated Environmental Monitoring Program (*CEMP*) is administered by the Oslo and Paris Commissions (OSPAR) in their effort to assess and remedy anthropogenic impact on the marine environment of the North East Atlantic. The Norwegian contribution to the *CEMP* was initiated by the Norwegian Climate and Pollution Agency in 1981 as part of the national monitoring program, and the current focus is on the levels, trends and effects of hazardous substances, including PCBs. It comprises several areas, including the Oslofjord and adjacent localities [15].

Objectives

The objective of this study was to elucidate the role of sediments as source of PCBs accumulated in Atlantic cod (*Gadus morhua*) through two exposure routes: (1) through (direct) diffusion from the sediment (via the water phase), and (2) through the food chain (dietary exposure). Furthermore, known PCB-concentrations in liver of cod from the inner Oslofjord, available through a national environmental monitoring program (*CEMP*; described above), were used as reference to assess the role of contaminated sediments specifically for the cod in the inner Oslofjord.

Current chemical legislation and regulating organs use a framework and criteria to assess the potential hazard and risk according to the chemicals’ bioaccumulative potential (B), in addition to their persistence (P) and toxicity (T) (“PBT” criteria; [e.g. 16]). These criteria are continuously

challenged [e.g. 8, 17]. Based on recent discussions among scientists and regulators, several recommendations have been put forward regarding evaluation of the B-criterion [e.g. 8]. These recommendations include taking into account the accumulation from the diet by the use of biomagnification factors (BMF; ratio between predator and prey concentrations) and/or trophic magnification factors (TMF; the average factor by which the lipid normalized concentration increases per trophic level; determined from the slope (m) derived by linear regression of Log_{10}-transformed biota concentration and trophic position; $TMF = 10^m$) when evaluating the bioaccumulation potential of a chemical. The present study also serves as a trial for the feasibility of such an approach.

As such, organisms used in the present study were Atlantic cod and the "King rag" worm *Nereis virens* (Polychaeta). The study has comprised two long term (months) mesocosm experiments:

1. Study of the bioaccumulation of PCBs in cod exposed to resuspended contaminated sediment particles ('the sediment resuspension experiment').
2. Study of the bioaccumulation of PCBs in cod fed benthic invertebrates (the polychaete *Nereis virens*) exposed to contaminated sediment ('the dietary exposure experiment').

In both exposure experiments, cod were exposed for a total of 129 days, with sampling at d 0, d 13, d 26, d 39, d 52, d 66, d 97 and d 129. In the latter experiment, the polychaetes were exposed to sediment for a minimum of 9 weeks before being fed to the cod.

The organisms employed were chosen for the commercial value, ecological relevance, the availability, and the experience that they are possible to hold in aquaria for extended periods.

Furthermore, Atlantic cod is also one of the species of choice in several environmental monitoring programs, including *CEMP*. The cod is common on the continental shelf in most of the North-Atlantic. Mostly, the cod is a benthic feeder, but may live pelagic. *Nereis virens* is common along the Atlantic coasts of Europe, North to the mid-West coast of Norway [18]. It occupies burrows in muddy sand. Sediment-dwelling organisms, such as several species in the *Nereis* genus are important prey items e.g. to demersal and bottom-feeding fish, such as cod, and may therefore contribute to the transport of contaminants to higher levels in marine food chains [e.g. 4].

The contaminated sediments employed in the experiments were from the inner Oslofjord, which includes the city harbor area of Oslo. The Norwegian Food Safety Authority has issued advice against consumption of cod liver from the inner Oslofjord, based on the PCB contamination.

In the present experiments, samples were also preserved for the evaluation of metabolites of polycyclic aromatic hydrocarbon (PAHs) in the bile of the fish, as well as for different biomarker responses. These will be discussed elsewhere (Daae et al. *in prep*.).

Materials and methods

Sediment sampling

The test-sediment (PCB-contaminated) was collected from the upper 5-15 cm of the sediments at locations in the Inner Oslofjord area (Eastern Norway), using a 0.1 m^2 Van Veen grab. The collection took place between 59° 52.176′ and 59° 53.974′ North and between 10° 40.630′ and

10° 43.682′ East. Uncontaminated reference (control) sediment was collected at a fixed location in the outer Oslofjord, previously employed in bioaccumulation studies and documented to have very low concentrations of organic pollutants [19]. The sediments were collected in spring, 2006.

For transport and prior to the experiments, the sediment was stored in 150-L boxes. Approximately 750 L of contaminated sediment (6 boxes) and 250 L of reference (control) sediment (2 boxes) were collected. The sediment was homogenized by shoveling aliquots of sediment between boxes simultaneously as they were slurried by the use of a mortar mixer for approximately 1 h (Eibenstock EHR-20 S, Elektrowerkzeuge GmBH Eibenstock, Germany).

Test-organisms

Atlantic cod were purchased from Marin Invest AS (Sandøy, Western Norway; resuspended sediment exposure experiment) and Marine Harvest ASA (Eggesbønes, Western Norway; dietary exposure experiment). The fish were brought to NIVA's marine research facility Solbergstrand by the use of tank lorries and held for a minimum of 2 months (acclimation) before initiation of the experiments. Prior to arrival, the fish were fed pellets: Gemma micro, Gemma 0.3/0.5, Gemma 0.75/1.0/1.2, Europa Respons 1.5 mm, Europa Respons 2.0 mm and Europa Respons 3.0 mm. After arrival at Solbergstrand, prior to the experiment, fish were fed Europa Respons 3.0 and 4.0 mm (supplier of all fish feed; Skretting AS, Stavanger, Norway). The experiments were conducted after approval by The Norwegian Animal Research Authority (NARA).

Rag worms (*Nereis virens*) were purchased from Seabait Ltd. (Ashington Northumberland, UK), and brought to NIVA's marine research facility Solbergstrand by air freight and car. Before

187 and during the experiments, the worms were fed Skretting advanced fish feed (Coapse fish - 23.
188 Skretting, Roman Island, Westfort Co., Mayo, Ireland).
189
190 *Experimental setup and sampling procedures*
191 The experimental procedures for 'the sediment resuspension experiment' were as follows:
192 Atlantic cod (approximately 450 g) were transferred to 6 fiberglass tanks (45 × 110 × 110 cm;
193 545 L) of which 3 tanks (the 'exposed' group) contained a 16 cm deep layer of sediment from the
194 inner Oslofjord (approximately 195 L of sediment in each tank; samples recovered for chemical
195 analysis). The remaining three tanks did not contain sediment ('control' group). At day zero (d 0;
196 March 3rd, 2006) 13 individual cod were transferred to each tank.
197
198 The tanks were supplied with running seawater (8 L min^{-1}; from 60 m depth outside the
199 research facility Solbergstrand). In this way the fish were ensured sufficient oxygen (measured to
200 75% saturation; WTW Oxi 340i; WTW GmbH, Weilheim, Germany). Through the exposure
201 period (129 days) the mean temperature was 7.4 °C (range: 6.3-9.2) and the mean salinity was
202 34.6 (range: 34.2-34.9; logged by WTW-probes, WTW GmbH). The fish were given a
203 maintenance diet (every second day) of pellets (3 mm and 4 mm; sampled for chemical analysis)
204 throughout the experiment to comply with their needs, but avoid excessive growth. Because of
205 the proportion of sediment in relation to amount water and fish, the swimming activity of the fish
206 could initially disturb the sediment sufficiently to produce turbid water. Mechanical disturbance
207 of the sediment was performed the last 4-5 weeks by the use of a small propeller (3 blades; ∅: 4
208 cm) mounted on a drill (Bosch P9B 600 RE; Robert Bosch AS, Ski, Norway). Sampling of fish
209 were performed at d 0, d 13, d 26, d 39, d 52, d 66, d 97 and d 129. Six fish were sampled at day

0. At every other outtake, one fish from each tank were sampled (n=3 in each group, ‘exposed’ and ‘control’). The fish were terminated by a blow to the head, before the gall-bladder was emptied of bile (using a syringe; handled elsewhere (Daae et al., *in prep*.)) and the liver was carefully excised and stored for chemical analysis (-20 °C; cod is a lean fish with the liver as the storage site for lipid reserves, thus nearly the whole body burden of lipophilic contaminants can be observed here [15]).

The experimental procedures for ‘the dietary exposure experiment’ were carried out in two phases, (1.) exposure of polychaetes to sediments and (2.) feeding polycheates to fish:

The exposure of polychaete worms was as follows: *N. virens* were exposed to the sediments (inner Oslofjord (‘exposed’) or outer Oslofjord (‘control’)) in containers of 11 L with lid. Approximately 8 L of sediments and 20-35 worms were added to each container, which was supplied with continuous water flow through (250 mL min^{-1}). One container was prepared for each feeding of fish (a total of 37 feedings). For logistical reasons, two rounds of polychaete exposure were conducted. Worms from the first exposure, were individually stored at -20 °C and served as ‘box lunch‘ for the fish towards the end of the fish exposure period (last 3 weeks). Furthermore, this batch functioned as the food backup, in case of unexpected mortality among the worms in the second batch. The worms from the second batch were extracted fresh from the sediment prior to each feeding of fish. Triplicate samples were prepared of sediments and polychaetes for chemical analysis.

The worms were fed pellets (see above, 2-3 g per container) 3 times each week, and were exposed to the sediments for a minimum of 9 weeks (which is twice the minimum duration recommended by Lee et al. [20]). Through the polychaete exposure periods the mean temperatures were 8.1 °C (range: 7.6-9.2) and 8.5 °C (range: 5.8-12.1), while the mean salinities were 34.3 (range: 33.9-34.5) and 34.1 (range: 33.4-34.5) for batch 1 and 2, respectively (logged by WTW-probes, WTW GmbH).

The feeding of sediment exposed-polychaetes to Atlantic cod was as follows: One week prior to the first feeding (d 0) the cod (mean weight: 78 g) were transferred to individual compartments in aquaria measuring 35 × 35 × 70 cm (3 compartments in each). One fish was added to each compartment. A total of 54 fish were thus occupying 18 aquaria. The aquaria were supplied air (bubbling) and continuous water flow through (1 L min^{-1}). Through the exposure period (129 days) the mean temperature was 7.7 °C (range: 6.6-9.7) and the mean salinity was 34.3 (range: 33.8-34.5; logged by WTW-probes, WTW GmbH).

The cod were fed exclusively *N. virens* twice a week (every 3rd to 4th day). The amount of worm (4-6 g) fed to the fish was weighed out and logged. The weekly amount of worm fed to the fish represented a minimum of 8% of the fish body weight. The individual compartments in the aquaria facilitated the individual feeding of the fish and at each feeding it was observed that the fish ingested all that was presented.

Sampling of fish was performed at d 0, d 13, d 26, d 39, d 52, d 66, d 97 and d 129. At d 0, six fish were sampled. At every other outtake, 3 fish were sampled from each group (fed worms

exposed to contaminated sediment ('exposed') or fed worms exposed to clean sediment ('control')). The fish were put to death by a blow to the head. At each sampling the fish length, weight and liver weight were measured. Samples were secured from the liver and stored (-20 °C) for chemical analysis. Furthermore, samples were preserved from bile, liver and blood for analysis of metabolites of polycyclic aromatic hydrocarbons (PAHs; in bile), activity of 7-ethoxyresorufin *O*-deetylase (EROD; in liver), amount of cytochrome P450 1A protein (CYP1A; in liver), amount of vitellogenin and zona radiata protein (in blood), and activity of δ-amino levulinic acid dehydrase (Ala-D; in blood). These biomarker responses are handled elsewhere (Daae et al., *in prep*.).

Extraction, cleanup and PCB analysis, and analysis of sediment properties

The chemical analyses were performed at NIVA. The laboratory is accredited by the Norwegian Accreditation as a testing laboratory according to the requirements of NS-EN ISO/IEC 17025 (2000). Furthermore, analytical standards are certified by the participation in international calibration tests, including QUASIMEME twice per year. The procedures for extraction, cleanup and quantification of PCB congeners were as described in Supplemental information, as are the analyses of sediment properties. The certified reference materials used were SRM 1944 and SRM 1588b (National Institute of Standards and Technology, Gaithersburg, MD, USA) and recoveries were 78 to 120 %. The detection limit was defined as >3 times signal noise and was from <0.05 to <1.0, dependent on congener and matrix.

Statistical methods

Statistical analysis was performed with the use of Statistica™ software (Ver 7.0; Statsoft,Tulsa, OK, USA). Temporal differences in cod liver PCB concentrations (within groups; "exposed" or "control") were evaluated using Analysis of Variance (ANOVA). Levene's test was used to test for heterogeneity of variance. If necessary, data were Log_{10}-transformed to reduce heterogeneity of variance. Furthermore, if homogeneity of variance was not obtained, temporal differences in PCB concentrations were evaluated using the non-parametric Kruskal-Wallis test, as were differences in PCB concentrations between cod exposed to contaminated sediment (directly or via polychaetes) and unexposed cod (no sediment exposure, or fed polychaetes exposed to clean control sediment), and differences in PCB concentrations between polychaetes exposed to contaminated sediments and polychaetes exposed to clean (control) sediments. The Dunnet post-hoc test (following ANOVA), or the non-parametric multiple comparison test (following Kruskal-Wallis), were employed to test for differences against zero-time. Linear regressions were applied to assess concentration increases in cod. A significance level of $\alpha = 0.05$ was chosen.

Results and Discussion

Methodical aspects

There was no mortality of cod during the exposure experiments, apart from one individual in the dietary exposure experiment (a surplus of fish was employed in the experiments (see above), thus this had no effect on the number of analyzed individuals). Apparently there was no, or minimal (not logged) mortality among the worms during the exposure, as there were plenty of worms in surplus for the feeding of cod, and no cadavers could be observed. The cod from the

dietary exposure experiment showed no signs of discomfort from a diet consisting exclusively of polychaetes. They soon became very tame, eating from the hand of the keeper. Furthermore, by day 129 of the exposure, they had gained 46.5% (mean ± 7.6 standard deviation) of their initial bodyweight (measured at d 0; corresponding to 33 g from a starting point of 71 g, on average), indicating that they were thriving on the worms. The holding of the fish, however (in terms of size of the setup) dictated limitations in the number of replicates (n=3).

The sediments applied in the two exposure experiments differed somewhat in PCB-content (see below; Table 1), despite the homogenization efforts (above). This renders direct comparisons between absolute concentrations accumulated in the fish from the two exposure experiments difficult. It should be noted, however, that the variability between replicates, within each experiment, was small. Direct comparisons between absolute concentrations accumulated in the fish from the two exposure experiments were further complicated by different lipid content (and different variability in such) of the fish livers, between exposure experiments (see below; Figure 1; Table S1, see Supplemental information).

It should also be noted that the because of the fairly high water flow-through (to meet the life support requirements of the fish) in the ‘sediment resuspension experiment’, the PCB distribution in the exposure system may not reflect partition equilibrium between sediment and water [21]. This may obscure the importance of PCB accumulation from sediment via the water phase. However, the flow-through conditions will resemble field conditions, where mixing and water movements will be present. On the other hand, resuspension of the sediment (to mimic disturbance of sediment in shallow waters) was done to facilitate desorption of particle associated PCBs and render them more available to the fish.

323

324 *Sediments and polychaetes*

325 Moderately high concentrations of PCBs were observed in the sediments used in the
326 experiments (Table 1; [22]), with concentrations a factor of ~4 higher in the dietary exposure
327 experiment than in the sediment resuspension experiment.

328

329 Concentrations of PCBs accumulated in *N. virens* were significantly higher in the exposed
330 worms than in the control group (a factor of 3 to 6; Table 1). The lipid content in the worms was
331 identical between groups. Calculating biota-to-sediment accumulation factors (BSAF;
332 $(C_{Org}/f_{Lip})/(C_{Sed}/f_{OC})$, where C_{Org} is the wet wt. concentration in the organism, f_{Lip} is the fraction of
333 tissue wet wt. that is lipid, C_{Sed} is the dry wt. concentration in the sediment, and f_{OC} is the fraction
334 of organic carbon in the sediment (g g^{-1} dry wt.)) gave values between 0.24 (PCB-28) and 0.67
335 (PCB-101). These values are somewhat lower (implying lower bioavailability) than a theoretical
336 expectation of 1.6 (see Supplemental information), provided the following assumptions [23]: (1.)
337 bioaccumulation of sediment associated PCBs in *N. virens* occurs (merely) as an equilibrium
338 partitioning between sediment particles (organic carbon in particular) and water, and between
339 water and the organism lipids, (2.) the relationship between the sediment:water partition
340 coefficient (K_d) and the organic carbon:water partition coefficient (K_{OC}) is $K_d = K_{OC} \times f_{OC}$, (3.)
341 There is a double logarithmic, linear relationship between K_{OC} and K_{OW} (the octanol:water
342 partition coefficient; $\log K_{OC} = \log K_{OW} - 0.21$; [24]; one domain sorption model) , and (4.) the
343 partitioning coefficient between the organism lipids and the water equals K_{OW}. Furthermore,
344 BSAFs of PCBs were somewhat lower than those e.g. observed in the oligochaete *Lumbriculus*
345 *variegatus* [25, 26]. On the other hand, BSAFs were orders of magnitude higher than those

observed for polycyclic aromatic hydrocarbons (PAHs) in e.g. *N. diversicolor* exposed to sediments with characteristic composition of sorption domains with high binding strength [23]. The values corresponded, however, well with previously observed BSAFs for PCBs in *N. diversicolor* [19] and grass shrimp (*Palaemonetes pugio*; [27]). The results indicate fairly high bioavailability of PCBs in the sediments, possibly slightly reduced by carbonaceous geosorbents present in the Oslofjord sediments [28].

Cod

Different lipid content in fish livers were (as mentioned) observed between exposure experiments (Figure 1; Table S1, see Supplemental information). Furthermore, the variability in lipid content among livers were different between exposure experiments (coefficient of variation, CV = 20.3% and 12.8% in the dietary exposure experiment and the sediment resuspension experiment, respectively; all individuals and sampling days). There were, however, no signs of a systematic change in lipid content, over time, in neither of the experiments, or groups (exposed vs. control); Figure 1; Table S1, see Supplemental information). Consequently, concentrations are treated/graphically expressed on a lipid wt. basis in the following (wet wt. concentrations presented in Table S1; see Supplemental information).

PCBs and other hydrophobic compounds express a high affinity for lipids [e.g. 7]. Ideally, equilibrium will eventually occur between the concentrations of these compounds in aquatic organisms and the surrounding water constituting their habitat [12]. Respiratory surfaces (i.e. gills) play an important role in this partitioning, as the compounds associate with the lipoid cell membranes in the gill epithelium and are circulated to lipid tissues within the organism. Equilibrium partitioning can be regarded as an approximate lipid:water partitioning, thus the K_{OW}

may provide valuable information [7]. The PCB congeners in focus of the present study have K_{OW} values ranging from 5.13 $\times 10^3$ (PCB-28) to 1.54 $\times 10^7$ (PCB-180), increasing with degree of chlorination [29].

An apparent increase in concentrations with time could be observed in the exposed group of the sediment resuspension experiment for most congeners (Figure 2). However, the hepatic concentrations of several congeners apparently also increased towards the end of the experiment in the control group (Figure 2). Nevertheless, significant differences were found between the exposed group and the control group, at several sampling days, but only for PCB-28 and -52 (those with the lowest K_{OW}; note limited statistical power due to low n). Furthermore, significantly different concentrations towards the end of the experiment, compared to d 0, were found for these congeners. The apparent increase, also in the control group, may likely be explained by fish in both groups being fed with commercial fish feed throughout the experiment. Analysis of this feed showed traces of PCBs (0.25 µg kg^{-1} (PCB-28 and -180) to 1.7 µg kg^{-1} (PCB-153) wet wt.; $\sum PCB_7$=6.75 µg kg^{-1} wet wt.; lipid content 16.0% wet wt.).

Ergo, the two congeners with the lowest hydrophobicity (K_{OW}) showed a temporal increase in concentrations, that may be related to accumulation of sediment associated PCBs, corresponding to previous observations [e.g. 27], suggesting lower bioavailability of higher chlorinated congeners in the water phase. According to Clark et al. [11], a large fraction of chemicals with K_{OW} $10^4 – 10^5$ may be present in the water phase (dissolved), when K_{OW}=10^6, half is adsorbed to particles present in the water, and when K_{OW}=10^8, all is adsorbed to particles. Furthermore,

several field observations suggest that aquatic organisms that accumulate PCBs from water (through diffusion), contain higher proportions of the lower chlorinated congeners [e.g. 3, 4].

The results further suggest that steady state is not reached (no indication of an asymptotic levelling) after 129 days for any of the congeners. Congeners with a lower degree of chlorination (and thus lower hydrophobicity) reach equilibrium faster than the higher chlorinated homologues [e.g. 30, 31]. An influence on the results by congener specific biotransformation by the fish can, however, not be ruled out.

There were markedly (statistically significant) higher concentrations of all PCB congeners in the exposed group, compared to the control, towards the end of the dietary exposure experiment (Figure 3). The PCB concentrations in the unexposed (control) group maintained a low level through the whole experiment (129 days; Figure 3). Significant differences in concentrations among sampling days and compared to d 0 could be observed (again) for congeners PCB-28 and -52 (significant differences among sampling days in the exposed group also for PCB-138 and -180; Figure 3; note low statistical power due to low n). Also in the dietary exposure experiment, there were no indications of an asymptotic levelling of the concentrations within the maximum exposure period of 129 days (Figure 3). Thus concentrations might very well have increased if the experiment was continued. This possible continued increase also illustrates challenges using biomagnification as a regulatory endpoint [8], if such potential must be shown prior to chemicals being released to the market and thus the environment (e.g. according to the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) of the European Union [16]). The Trophic Magnification Factor (TMF) is suggested as a “golden standard” in bioaccumulation and has been applied in many field studies [e.g. 8]. The present accumulation results, however,

suggests inappropriately complex, time consuming and expensive test protocols if TMFs would be applied to laboratory experiments, in terms of preventive environmental safety. Thus, the use of alternative approaches, such as measuring uptake and elimination rates (in an uptake phase and a subsequent depuration phase), to derive "steady-state biomagnification factors" [e.g. 8] seems more applicable in this regard.

Crude estimates of the assimilation efficiency of the PCBs fed to cod, through the polychaete "vehicle", during the 129 d exposure period could be made since the following parameters were known: (1.) the total amount (kg) polychaetes fed to the cod (2.) the mean PCB concentrations (μg kg^{-1}) in the polychaetes, (3.) initial (d 0) PCB concentrations (μg kg^{-1}) and weight (kg) of cod livers, (4.) terminal PCB concentrations (μg kg^{-1}) and weight (kg) of cod livers. The results show that 30-50% of the total amount of PCBs fed to the cod (via *N. virens*) through the 129 d exposure period is stored in the cod liver (Table S2; see Supplemental information). The highest assimilation efficiency was apparent for the lower chlorinated congeners (PCB-28 and -52).

According to Kelly et al. [32], the assimilation efficiency of different persistent organic compounds in fish is slightly less than 50% and decrease for compounds with $K_{OW}>10^7$. It is suggested that transport of very hydrophobic compounds across the intestinal wall is limited by an aqueous diffusion resistance [33]. Thus, a possible explanation for the decrease in dietary assimilation efficiency with increasing hydrophobicity, is slow transport through intestinal aqueous phases because of low aqueous solubility [34, 35]. An influence on the results by congener specific biotransformation by the fish can, however, not be ruled out.

As mentioned, there are factors that impede direct comparisons between the results of the sediment resuspension experiment and the dietary exposure experiment. Firstly, the sediment applied in the dietary exposure experiment contained somewhat higher concentrations of PCBs, than the sediment applied in the sediment resuspension experiment (Table 1). Secondly, there were differences in the liver lipid content of the fish employed in the two experiments (Figure 1; Table S1, see Supplemental information). In a review of bioaccumulation mechanisms and models, Mackay and Fraser [7] present a "rule of thumb" implying that the importance of dietary accumulation versus diffusive accumulation (across respiratory surfaces) is approximately K_{OW}/200 000. This relationship will vary dependent on fish size, condition and species. However, for very hydrophobic substances (i.e. log K_{OW}>6.5) diffusive uptake over respiratory surfaces will not be important, while for less hydrophobic substances (i.e. log K_{OW}<4.0), dietary uptake becomes less important, since equilibrium between the fish and the surrounding water will be reached more quickly. The results of the present study (considering the above mentioned complicating factors, however) do not suggest this "rule of thumb" erroneous.

Extrapolations and concluding remarks

In the dietary exposure experiment, higher concentrations were observed in the exposed group, compared to the control towards the end of the exposure period (d 52 – d 129) for all congeners (Figure 3). Furthermore, no increases in concentrations were indicated in the control group (Figure 3). Plotting time (days; continuous scale) versus concentration (exposed group), produced significant (p<0.0014) linear regressions for all congeners (as well as $\sum PCB_7$; Figure S1, see Supplemental information). The goodness-of-fit decreased, however, for the more chlorinated/hydrophobic congeners (R^2= 0.76, 0.68, 0.40, 0.39, 0.34, 0.44, 0.44 and 0.43 for

462 PCB-28, -52, -101, -118, -153, -138, -180 and $\sum PCB_7$, respectively; Figure S1, see Supplemental
463 information). Given the following assumptions: (1.) a continued linear increase in concentrations
464 and (2.) an initial concentration equal to the intercept of the regression (approximately the
465 medians of the d 0 concentrations; see Figure S1, Supplemental information), the slopes of the
466 regressions may be used to make crude estimates/extrapolations of the time needed to reach
467 concentrations present in wild caught cod from the inner Oslofjord (known through
468 environmental monitoring; Table 2). Such extrapolations showed that the time needed to reach
469 concentrations present in wild Oslofjord cod were 0.2 (PCB-28) to 5.8 (PCB-153) years (Table
470 3). It must be noted that these extrapolations may likely represent underestimates, since the
471 assumption of a continued linear increase until reaching concentrations present in wild Oslofjord
472 cod might be erroneous. Alternatively, the increase might be curvilinear (first order; [e.g. 36,
473 37]). Additionally, the issue of growth dilution must be taken into account. For compounds with
474 concentrations that change slowly, a growth constant of e.g. 0.001 Day^{-1} (corresponding to a
475 doubling in size in slightly less than 2 years) will lead to a considerable dilution in the organism
476 [7]. Other factors will also increase the uncertainty of such crude extrapolations. Wild cod also
477 feed on other organisms than polychaetes [e.g. 38], and at a certain size, a shift in trophic position
478 may occur. Furthermore, the PCB concentrations of the Oslofjord sediment are obviously not
479 uniform [e.g. 39] and will be both higher and lower than those used in the experiment in some
480 areas. Nevertheless, generally the results suggest that the contaminated sediments of the inner
481 Oslofjord are an important source of legacy PCBs for accumulation in the native cod, although
482 additional contributions from e.g. atmospheric deposition and runoff from the surrounding
483 (urban) landscapes also may be substantial [40]. The study has further indicated the feasibility of
484 conducting long term (months) experiments for elucidating contaminant accumulation from

sediments to fish, via one level of the food chain, providing opportunities for related topics. On the other hand, challenges for applying Trophic Magnification Factors (TMF) to determine biomagnification in laboratory experiments, in terms of preventive environmental safety, are indicated. The results will provide useful information for parameterization of models describing the behaviour of hydrophobic persistent contaminants in the foodweb of the Oslofjord and elsewhere.

Supplemental information

Extraction, cleanup and PCB analysis, Sediment property analyses, Table S1, Table S2, Figure S1, Calculation of biota-to-sediment accumulation factors (BSAFs).

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623 **Figure Legends**

624

625 **Figure 1.** Lipid content (% wet wt.) in liver of cod (*Gadus morhua*) from the sediment
626 resuspension experiment (left) and the dietary exposure experiment (right) after 13, 26, 39, 52,
627 66, 97 and 129 days; n=3 at all sample days (and both groups; exposed vs. control), except at d 0,
628 where n=6. Median, minimum and maximum are depicted (i.e. all observations, except at d 0). In
629 the sediment resuspension experiment, the 'exposed' fish were experimentally exposed to
630 resuspended sediment from the inner Oslofjord, while the 'control' fish were not exposed to
631 sediment. In the dietary exposure experiment, the 'exposed' fish were fed polychaetes (*Nereis*
632 *virens*) previously exposed to sediment from the inner Oslofjord, while the 'control' fish were fed
633 *N. virens* previously exposed to unpolluted sediment. Note: Categorical X-axis.

634

635 **Figure 2.** Concentrations ($\mu g\ kg^{-1}$; lipid wt.) of PCBs (-28 , -52, -101, -118, -153, -138 and -180,
636 and the sum of these, $\sum PCB_7$) in liver of cod (*Gadus morhua*) from the sediment resuspension
637 experiment after 13, 26, 39, 52, 66, 97 and 129 days; n=3 at all sample days (and both groups;
638 exposed vs. control), except at d 0, where n=6. Median, minimum and maximum are depicted
639 (i.e. all observations, except at d 0). The 'exposed' fish were experimentally exposed to
640 resuspended sediment from the inner Oslofjord, while the 'control' fish were not exposed to
641 sediment. Significant differences between 'exposed' and 'control' are indicated by "*****".
642 Significant differences among sampling days in the exposed group are indicated by "**a**", while
643 significant differences among sampling days in the control group are indicated by "**b**".
644 Significant differences between each specific sampling day and d 0 are indicated by "**c**". Note:
645 different scale on response axes; categorical X-axis.

646

647 **Figure 3.** Concentrations (μg kg^{-1}; lipid wt.) of PCBs (-28 , -52, -101, -118, -153, -138 and -180,
648 and the sum of these, $\sum PCB_7$) in liver of cod (*Gadus morhua*) from the dietary exposure
649 experiment after 13, 26, 39, 52, 66, 97 and 129 days; n=3 at all sample days (and both groups;
650 exposed vs. control), except at d 0, where n=6. Median, minimum and maximum are depicted
651 (i.e. all observations, except at d 0). The 'exposed' fish were fed polychaetes (*Nereis virens*)
652 previously exposed to sediment from the inner Oslofjord, while the 'control' fish were fed *N.*
653 *virens* previously exposed to unpolluted sediment. Significant differences between 'exposed' and
654 'control' are indicated by "*". Significant differences among sampling days in the exposed group
655 are indicated by "**a**", while significant differences among sampling days in the control group are
656 indicated by "**b**". Significant differences between each specific sampling day and d 0 are
657 indicated by "**c**". Note: different scale on response axes; categorical X-axis.

658

Figure 1.

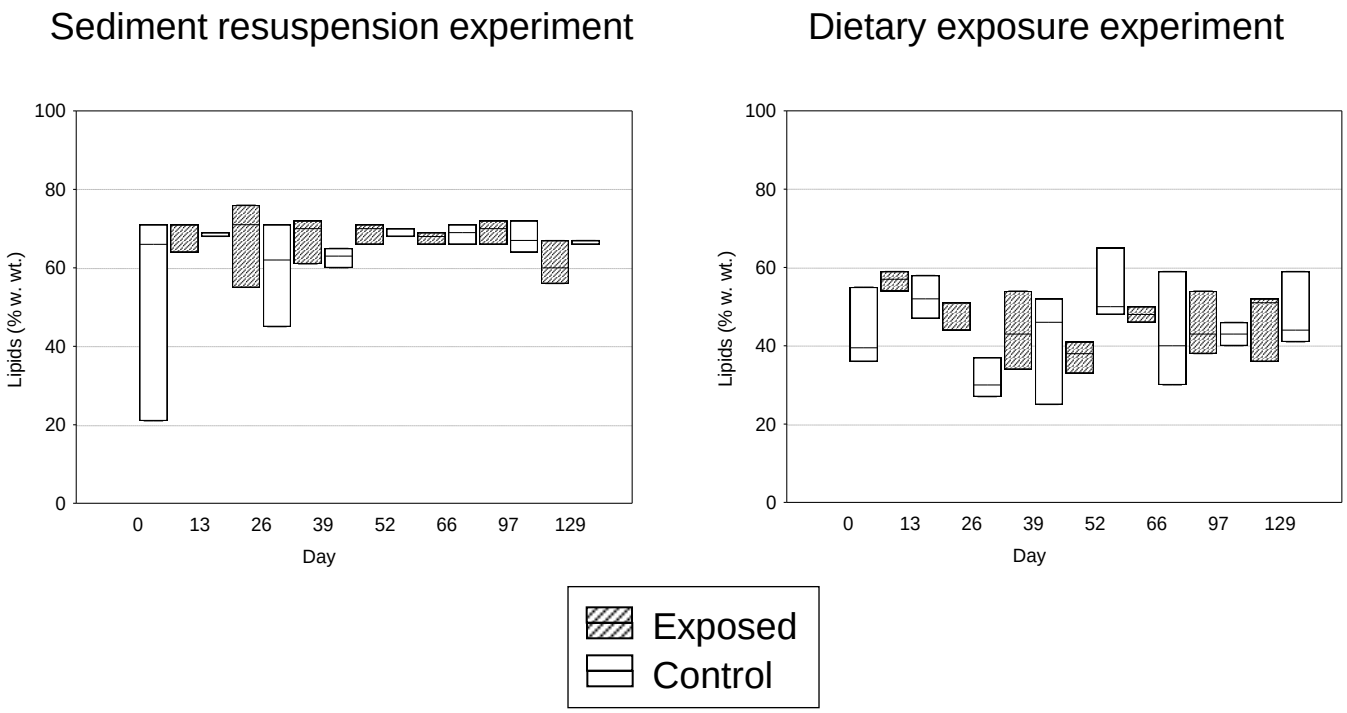
Sediment resuspension experiment
Dietary exposure experiment
Lipids (% w. wt.)
100
80
60
40
20
0
0
13
26
39
52
66
97
129
Day
Exposed
Control

Figure 2.

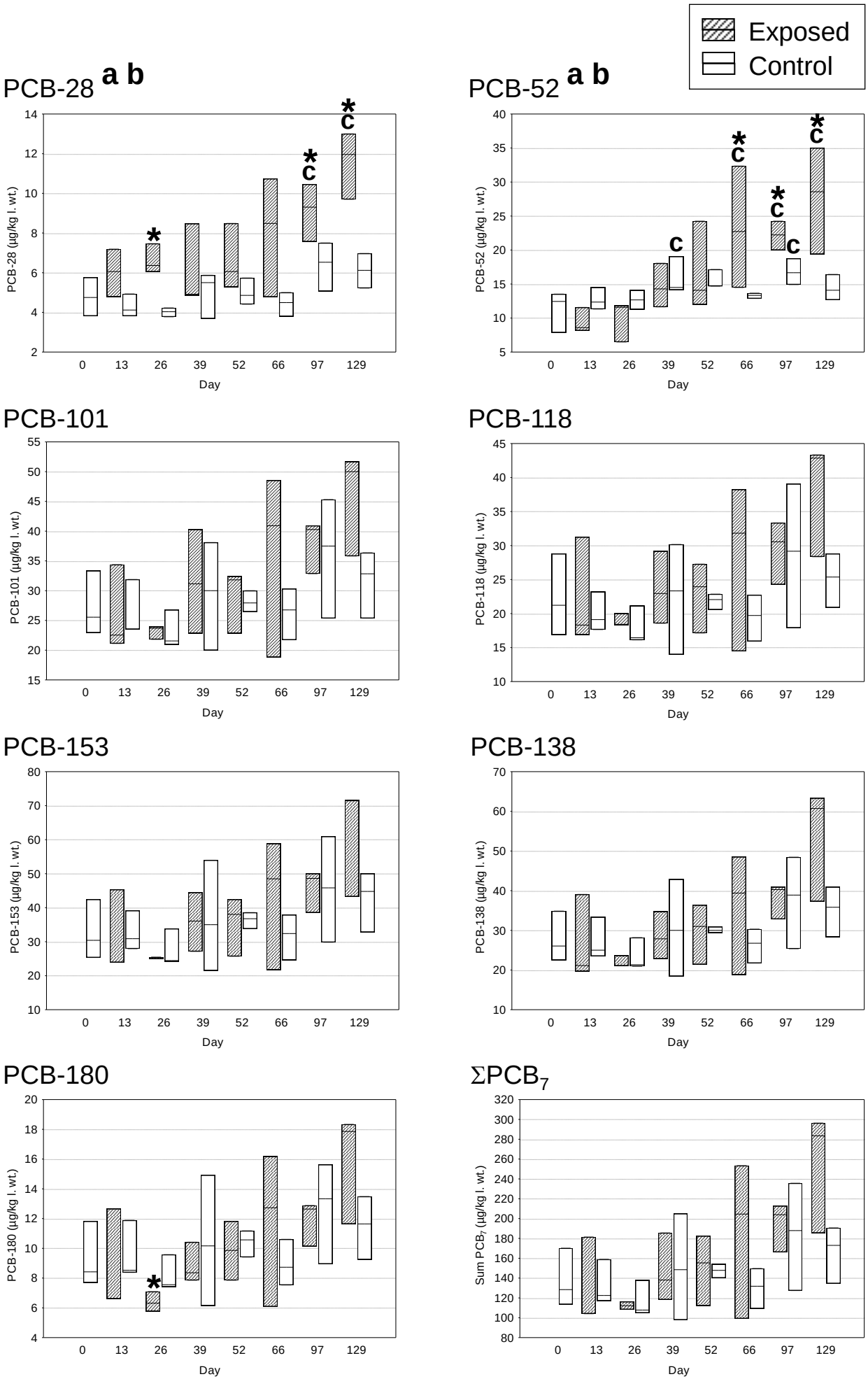
Exposed
Control
PCB-28 a b
PCB-52 a b
PCB-101
PCB-118
PCB-153
PCB-138
PCB-180
ΣPCB7
PCB-28 (µg/kg l. wt.)
PCB-52 (µg/kg l. wt.)
PCB-101 (µg/kg l. wt.)
PCB-118 (µg/kg l. wt.)
PCB-153 (µg/kg l. wt.)
PCB-138 (µg/kg l. wt.)
PCB-180 (µg/kg l. wt.)
Sum PCB7 (µg/kg l. wt.)
Day
0 13 26 39 52 66 97 129

Figure 3.

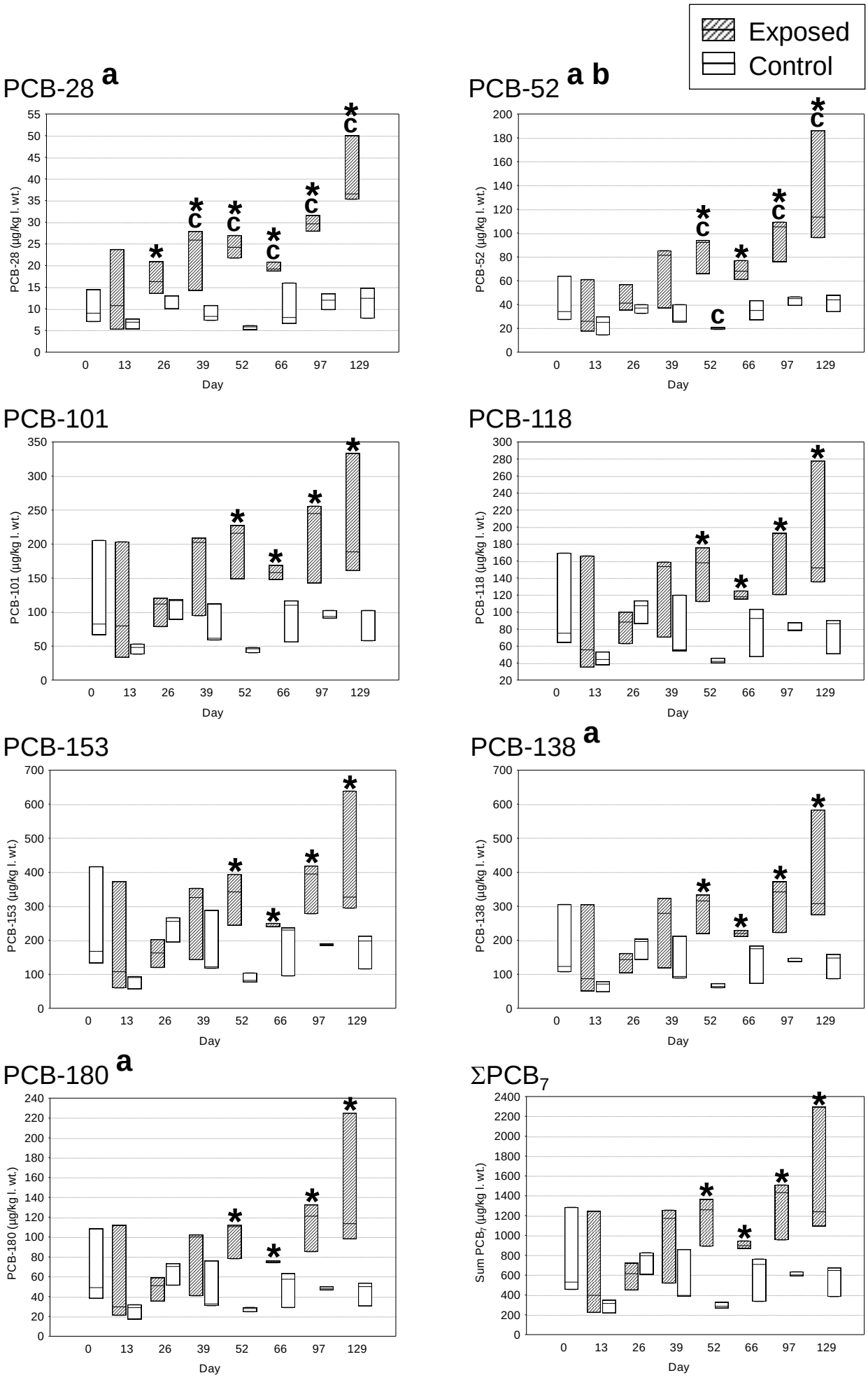
Exposed
Control
PCB-28 a
PCB-52 a b
PCB-101
PCB-118
PCB-153
PCB-138 a
PCB-180 a
ΣPCB7
PCB-28 (µg/kg l. wt.)
PCB-52 (µg/kg l. wt.)
PCB-101 (µg/kg l. wt.)
PCB-118 (µg/kg l. wt.)
PCB-153 (µg/kg l. wt.)
PCB-138 (µg/kg l. wt.)
PCB-180 (µg/kg l. wt.)
Sum PCB7 (µg/kg l. wt.)
Day
0 13 26 39 52 66 97 129

Table 1. Amount dry matter (% wet wt.), amount of particles larger than 63 µm (% dry wt.), total amount of organic carbon (TOC; % dry wt.) and concentrations of PCB-congeners (µg kg^{-1}, dry wt.) in sediments used in the sediment resuspension experiment and the dietary exposure experiment, and lipid content (% wet wt.) and concentrations of PCB-congeners (µg kg^{-1}, wet wt.) in polychaetes (*Nereis virens*) exposed to contaminated (exposed) and reference (control) sediment in the dietary exposure experiment.

Experiment	Matrix (group)	dry matter (% w. wt.)	> 63 µm (% d. wt.)	TOC (% d. wt.)	PCB-28 (µg kg^{-1})	PCB-52 (µg kg^{-1})	PCB-101 (µg kg^{-1})	PCB-118 (µg kg^{-1})	PCB-153 (µg kg^{-1})	PCB-138 (µg kg^{-1})	PCB-180 (µg kg^{-1})	ΣPCB_7 (µg kg^{-1})
Sed. resusp.	**Sediment (exposed)**	45.0 (44.0-45.0)	88.0 (85.0-89.0)	3.2 (2.8-4.1)	0.80 (0.71-0.91)	1.1 (0.92-1.2)	1.5 (1.1-1.6)	1.2 (1.0-1.3)	2.0 (1.5-2.1)	2.5 (1.9-2.6)	0.92 (0.71-0.98)	10.0 (7.8-10.7)
Dietary exposure	**Sediment (exposed)**	40.6 (39.6-51.5)	78.2 (70.0-80.7)	3.0 (3.0-3.1)	2.8 (2.7-2.9)	4.3 (3.8-4.3)	4.8 (4.4-4.9)	5.5 (4.9-5.5)	11 (11-12)	8.2 (7.7-8.4)	4.4 (3.9-4.5)	41.3 (38.4-42.2)
	Sediment (control)	74.8 (73.3-75.1)	74.2 (72.1-78.2)	0.7 (0.5-1.0)	<0.5	- *	<0.5	<0.5	<0.5	<0.5	<0.5	n.d.

Experiment	Matrix (group)	lipids (% w. wt.)	PCB-28 (µg kg^{-1})	PCB-52 (µg kg^{-1})	PCB-101 (µg kg^{-1})	PCB-118 (µg kg^{-1})	PCB-153 (µg kg^{-1})	PCB-138 (µg kg^{-1})	PCB-180 (µg kg^{-1})	ΣPCB_7 (µg kg^{-1})
Dietary exposure	**Polych. (exposed)**	2.8 (2.2-4.3)	0.66 (0.54-0.71)	1.6 (1.3-2.3)	2.8 (2.6-3.6)	2.3 (2.1-2.5)	4.4 (3.9-5.5)	4.0 (3.6-5.1)	1.6 (1.4-1.9)	17.4 (15.4-21.6)
	Polych. (control)	2.8 (2.3-4.4)	0.12 (0.08-0.13)	0.42 (0.24-0.55)	0.81 (0.75-0.94)	0.82 (0.77-0.84)	1.6 (1.4-1.6)	1.3 (1.2-1.3)	0.46 (0.45-0.47)	5.5 (5.1-5.7)

* Coelution in chromatogram.

Table 2. Lipid content (% wet wt.) and concentrations (µg kg^{-1}; wet wt. and lipid wt., respectively) of PCBs (-28 , -52, -101, -118, -153, -138 and -180, and the sum of these, $\sum PCB_7$) in liver of native cod (*Gadus morhua*) from the inner Oslofjord sampled (autumn) each year 2000-2006 through the Coordinated Environmental Monitoring Program (*CEMP*; [15]). Mean and (standard deviation) is presented, n=175 (25 individuals each year).

	Lipid (%)	**PCB-28 (µg kg^{-1})**	**PCB-52 (µg kg^{-1})**	**PCB-101 (µg kg^{-1})**	**PCB-118 (µg kg^{-1})**	**PCB-153 (µg kg^{-1})**	**PCB-138 (µg kg^{-1})**	**PCB-180 (µg kg^{-1})**	**ΣPCB_7 (µg kg^{-1})**
Wet wt.	38.3 (16.8)	10.1 (7.8)	49.1 (46.7)	195.7 (150.7)	429.3 (221.8)	1154.0 (588.0)	799.7 (373.7)	317.8 (165.0)	2795.9 (1374.8)
Lipid wt.	-	26,0 (16.0)	128.3 (87.2)	563.5 (383.5)	1365.7 (991.3)	3932.8 (3316.6)	2670.7 (2171.4)	1096.8 (900.6)	9255.0 (7203.3)

Table 3. *Slope* (μg kg^{-1} Day^{-1}) of linear regressions (see Figure S1, Supplemental information): Day vs. PCB concentrations from the exposed group of the dietary exposure experiment, where fish were fed polychaetes (*Nereis virens*) previously exposed to sediment from the inner Oslofjord, as well as **number of days (and years)** to reach the concentrations that are observed in wild caught fish from the inner Oslofjord (see Table 2). Assumptions: continued linear increase (extrapolation), initial fish concentrations equal the intercept of the regressions (approximately median at d 0; see text and Figure S1, Supplemental information), no growth dilution, a strict polychaete diet and uniform PCB concentrations in the Oslofjord sediments.

	PCB-28	**PCB-52**	**PCB-101**	**PCB-118**	**PCB-153**	**PCB-138**	**PCB-180**	**ΣPCB_7**
Slope	*0.2193*	*0.7133*	*1.0591*	*0.8103*	*1.7664*	*1.8290*	*0.6727*	*7.0701*
Days to reach Oslofjord level	**71**	**133**	**434**	**1581**	**2123**	**1383**	**1556**	**1223**
(Years to reach Oslofjord level)	**(0.2)**	**(0.4)**	**(1.2)**	**(4.3)**	**(5.8)**	**(3.8)**	**(4.3)**	**(3.4)**

SUPPLEMENTAL INFORMATION

Accumulation of polychlorinated biphenyls from contaminated sediment by Atlantic cod (*Gadus morhua*) – direct accumulation from resuspended sediment and dietary accumulation via the polychaete *Nereis virens*

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Extraction, cleanup and PCB analysis

The procedures for extraction, cleanup and quantification of PCB congeners were as follows:

Samples of cod liver or polychaetes were homogenized, using an Ultra Turrax™ (Ika-Werke GmbH, Staufen, Germany), and added internal standards (50 ng each of PCB-30, -53 and -204). The PCBs were extracted twice with cyclohexane and acetone (4:3, vol:vol) by ultrasonication for 3 to 5 minutes. The extracts were subsequently washed with saline solution (0.5%) before the extraction volume was reduced and the solvent exchanged to dichloromethane. After cleanup by gel permeation chromatography (GPC), the solvent was exchanged to cyclohexane. Further cleanup of the extracts was performed by treatment with concentrated sulphuric acid. Aliquots of the lipid extracts were used to gravimetrically determine the lipid content. Samples of the extracts were injected automatically on a gas chromatograph with electron capture detection (GC/ECD; HP/Agilent 5890; Agilent Technologies, Wilmington, DE, USA). The concentrations of the standard solutions were in the range 2-1000 ng μl^{-1}. The GC was equipped with a 60m J&W column with a stationary phase of 5% phenyl polysiloxane (0.25 mm i.d. and 0,25 µm film thickness; J&W Scientific, Folsom, CA, USA), and an inlet operated in the splitless mode. The initial column temperature was 90 °C, which after two minutes was raised to 180 °C at a rate of 10 °C min^{-1}, thereafter raised to 270 °C at a rate of 2 °C min^{-1}. Then the temperature was raised to 310 °C at a rate of 20 °C min^{-1}. The injector temperature was 255 °C, the detector temperature 285 °C and the column flow rate was 1 ml min^{-1}. H_2 was used as carrier gas (1 ml min^{-1}) and N_2 was used as make-up gas (30 ml min^{-1}; AGA, Oslo, Norway). The GC was connected to a H.P. Compaq Pentium D PC equipped with the software program GC-Chemstation Rev.B.02.01 (Agilent Technologies) for integration purposes. The individual PCB congeners were determined (peak height) against corresponding components in standards obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Sediment samples were homogenized and added internal standards. The PCBs were extracted with dichloromethane and cyclohexane (1:1 vol:vol) by Accellerated Solvent Extraction (ASE-200; Dionex Corp., Sunnyvale, CA, USA) at a temperature of 100 °C and a pressure of 2000 psi. Cleanup and GC/MS analysis was performed as described above.

Sediment property analyses

The organic content of the sediments were determined after combustion at 1800 °C in a Carlo Erba 1106 elemental analyser (Thermo Electron Corp., Milan, Italy). TOC was determined by acidification to remove inorganic carbon and reanalysis of the remaining total carbon. The particle fractions were measured according to Krumbein and Pettijohn [1].

Table S1. Lipid content (% wet wt.) and concentrations (µg kg^{-1}; wet wt.) of PCBs (-28 , -52, -101, -118, -153, -138 and -180, and the sum of these, ∑PCB_7) in liver of cod (*Gadus morhua*) from the sediment resuspension experiment (**a.**) and the dietary exposure experiment (**b.**) after 13, 26, 39, 52, 66, 97 and 129 days; n=3 at all sample days (and both groups; exposed vs. control), except at d 0, where n=6. Median and range are presented (i.e. all observations, except at d 0). In the sediment resuspension experiment, the 'exposed' fish were experimentally exposed to resuspended sediment from the inner Oslofjord, while the 'control' fish were not exposed to sediment. In the dietary exposure experiment, the 'exposed' fish were fed polychaetes (*Nereis virens*) previously exposed to sediment from the inner Oslofjord, while the 'control' fish were fed *N. virens* previously exposed to unpolluted sediment.

a.

Group	Day	Lipids (% w. wt.)	PCB-28 (µg kg^{-1})	PCB-52 (µg kg^{-1})	PCB-101 (µg kg^{-1})	PCB-118 (µg kg^{-1})	PCB-153 (µg kg^{-1})	PCB-138 (µg kg^{-1})	PCB-180 (µg kg^{-1})	ΣPCB_7 (µg kg^{-1})
zero (control)	**0**	66	2.8	7.8	16.5	12.5	18.5	16	5.4	80.8
		(21-71)	(1.1-3.8)	(2.6-9.6)	(5.7-22)	(4.9-19)	(6.9-28)	(6-23)	(1.9-7.8)	(29.1-112.3)
Exposed	**13**	71	4.3	6.1	16	13	17	15	4.7	74.1
		(64-71)	(3.4-4.6)	(5.8-7.4)	(15-22)	(12-20)	(17-29)	(14-25)	(4.7-8.1)	(73.9-116.1)
	26	71	4.6	6.5	17	13	18	15	4.1	77
		(55-76)	(3.5-5.3)	(4.6-8.8)	(12-18)	(11-14)	(14-19)	(13-16)	(3.9-4.8)	(63.9-85.2)
	39	70	3.4	10	19	14	22	17	5.5	84.2
		(61-72)	(3-6.1)	(7.1-13)	(16-29)	(13-21)	(19-32)	(16-25)	(5.1-7.5)	(82.9-133.6)
	52	70	4.3	10	21	17	27	22	7	110.3
		(66-71)	(3.7-5.6)	(8.4-16)	(16-23)	(12-18)	(18-28)	(15-24)	(5.5-7.8)	(78.6-120.4)
	66	68	5.6	15	27	21	32	26	8.4	135
		(66-69)	(3.3-7.3)	(10-22)	(13-33)	(10-26)	(15-40)	(13-33)	(4.2-11)	(68.5-172.3)
	97	70	6.7	16	27	22	33	27	8.5	140.4
		(66-72)	(5.3-6.9)	(14-16)	(23-29)	(17-22)	(27-35)	(23-29)	(7.1-9.1)	(116.4-146.8)
	129	60	6.7	16	28	24	40	34	10	158.7
		(56-67)	(6.5-7.8)	(13-21)	(24-31)	(19-26)	(29-43)	(25-38)	(7.8-11)	(124.3-177.8)

Table continued on text page.

Table 1. a. continued

Group	Day	Lipids (% w. wt.)	PCB-28 (µg kg^{-1})	PCB-52 (µg kg^{-1})	PCB-101 (µg kg^{-1})	PCB-118 (µg kg^{-1})	PCB-153 (µg kg^{-1})	PCB-138 (µg kg^{-1})	PCB-180 (µg kg^{-1})	ΣPCB$_7$ (µg kg^{-1})
Control	**13**	68	2.8	8.4	16	13	21	17	5.8	83.3
		(68-69)	(2.6-3.4)	(7.7-10)	(16-22)	(12-16)	(19-27)	(16-23)	(5.7-8.2)	(79.7-109.6)
	26	62	2.5	7	13	10	15	13	4.6	65.1
		(45-71)	(1.7-3)	(5.7-10)	(9.7-19)	(7.4-15)	(11-24)	(9.6-20)	(3.4-6.8)	(48.5-97.8)
	39	63	3.3	9.2	18	14	21	18	6.1	89.1
		(60-65)	(2.4-3.7)	(8.7-12)	(13-24)	(9.1-19)	(14-34)	(12-27)	(4-9.4)	(63.7-129.1)
	52	68	3.4	10	19	15	25	21	7.4	100.5
		(68-70)	(3-3.9)	(10-12)	(18-21)	(14-16)	(23-27)	(20-21)	(6.4-7.6)	(95.4-107.8)
	66	69	3.1	9.2	19	14	23	19	6.2	93.6
		(66-71)	(2.7-3.3)	(8.5-9.7)	(15-20)	(11-15)	(17-25)	(15-20)	(5.2-7)	(75.5-98.8)
	97	64	4.7	12	27	21	33	28	9.6	135.3
		(64-72)	(3.4-4.8)	(10-12)	(17-29)	(12-25)	(20-39)	(17-31)	(6-10)	(85.4-150.8)
	129	67	4.1	9.3	22	17	30	24	7.8	115.9
		(66-67)	(3.5-4.6)	(8.5-11)	(17-24)	(14-19)	(22-33)	(19-27)	(6.2-8.9)	(90.2-125.8)

b.

Group	Day	Lipids (% w. wt.)	PCB-28 (µg kg^{-1})	PCB-52 (µg kg^{-1})	PCB-101 (µg kg^{-1})	PCB-118 (µg kg^{-1})	PCB-153 (µg kg^{-1})	PCB-138 (µg kg^{-1})	PCB-180 (µg kg^{-1})	ΣPCB$_7$ (µg kg^{-1})
zero (control)	**0**	39.5 (36-55)	4 (2.6-5.2)	14.5 (12-23)	38 (28-74)	33.5 (27-61)	72.5 (56-150)	55.5 (45-110)	21 (17-39)	239.6 (190.9-462.2)
Exposed	**13**	57 (54-59)	5.8 (3-14)	14 (10-36)	43 (19-120)	30 (20-98)	58 (34-220)	47 (29-180)	16 (12-66)	213.8 (127.0-734.0)
	26	51 (44-51)	8.3 (6.9-9.2)	21 (18-25)	53 (40-57)	44 (32-45)	83 (61-89)	71 (53-73)	26 (18-26)	313.3 (228.9-317.2)
	39	43 (34-54)	8.8 (7.7-12)	29 (20-35)	71 (51-87)	54 (38-66)	120 (77-140)	110 (64-120)	34 (22-44)	426.8 (279.7-504.0)
	52	38 (33-41)	8.9 (8.9-9.2)	31 (27-35)	75 (61-82)	58 (46-60)	130 (100-130)	110 (90-120)	37 (32-42)	449.9 (364.9-478.2)
	66	48 (46-50)	9.6 (8.6-10)	34 (28-37)	79 (68-81)	59 (53-60)	120 (110-120)	110 (97-110)	36 (35-37)	448.6 (399.6-454.0)
	97	43 (38-54)	12 (12-16)	41 (40-47)	93 (77-110)	73 (65-83)	150 (150-180)	130 (120-160)	46 (46-57)	544.0 (515.0-649.0)
	129	51 (36-52)	18 (18-19)	59 (49-67)	98 (82-120)	79 (69-100)	170 (150-230)	160 (140-210)	59 (50-81)	644.0 (558.0-826.0)

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Table 1. b. continued

Group	Day	Lipids (% w. wt.)	PCB-28 (µg kg^{-1})	PCB-52 (µg kg^{-1})	PCB-101 (µg kg^{-1})	PCB-118 (µg kg^{-1})	PCB-153 (µg kg^{-1})	PCB-138 (µg kg^{-1})	PCB-180 (µg kg^{-1})	ΣPCB$_7$ (µg kg^{-1})
Control	**13**	52	3.6	13	25	23	44	37	15	163.6
		(47-58)	(3.1-3.6)	(8.3-14)	(22-25)	(22-25)	(33-47)	(28-37)	(10-15)	(126.4-163.6)
	26	30	3.7	12	33	32	72	53	19	224.7
		(27-37)	(2.7-3.9)	(10-12)	(32-35)	(29-34)	(69-80)	(53-61)	(19-22)	(214.7-247.9)
	39	46	3.8	12	28	29	61	46	16	200.8
		(25-52)	(2.7-3.8)	(10-13)	(27-32)	(25-30)	(56-72)	(43-53)	(15-19)	(181.8-214.7)
	52	50	2.8	10	23	22	50	35	14	156.8
		(48-65)	(2.6-4)	(9.5-13)	(23-26)	(21-26)	(41-50)	(32-39)	(14-16)	(143.1-174.0)
	66	40	3.9	14	35	31	71	55	19	228.8
		(30-59)	(3.2-4.8)	(13-16)	(33-44)	(28-37)	(56-92)	(43-70)	(17-23)	(196.9-283.2)
	97	43	4.8	18	41	35	81	59	20	258.8
		(40-46)	(4.5-5.8)	(18-20)	(39-43)	(34-36)	(76-85)	(59-63)	(20-22)	(253.8-271.5)
	129	44	5.1	20	42	37	87	65	22	276.1
		(41-59)	(4.6-6.5)	(18-21)	(34-45)	(30-38)	(68-87)	(51-65)	(18-22)	(225.6-284.5)

Table S2. PCBs accumulated in liver of cod (*Gadus morhua*) after a 129 days exposure period (where they were fed twice a week with *Nereis virens* previously exposed to PCB-polluted sediment from the inner Oslofjord). Mean percentage (%; and standard deviation, *s*) of the total amount of PCBs fed to cod (via the *N. virens* "vehicle"), and Log K_{OW} [2] is presented.

By day 129:	**PCB-28**	**PCB-52**	**PCB-101**	**PCB-118**	**PCB-153**	**PCB-138**	**PCB-180**	**ΣPCB_7**
Accumulated (mean %)	50	49	37	32	41	36	30	38
s	27.3	26.5	20.2	16.6	19.0	17.2	16.5	18.7
(Log K_{OW})	(5.7)	(5.8)	(6.3)	(6.7)	(6.7)	(6.7)	(7.2)	

Figure S1. Linear regressions: time (days) vs. concentrations (µg kg^{-1} lipid wt.) of PCBs (-28, -52, -101, -118, -153, -138 and -180, and the sum of these, $\sum PCB_7$, respectively) in liver of 'exposed' cod (*Gadus morhua*) from the dietary exposure experiment (sampling after 13, 26, 39, 52, 66, 97 and 129 days; n=3 at all sample days, except at d 0, where n=6). In the dietary exposure experiment, the 'exposed' fish were fed polychaetes (*Nereis virens*) previously exposed to sediment from the inner Oslofjord. Equations and R^2 for the regression lines are indicated. All regressions were statistically significant (P<0.0014). Note: different scales on concentration axes.

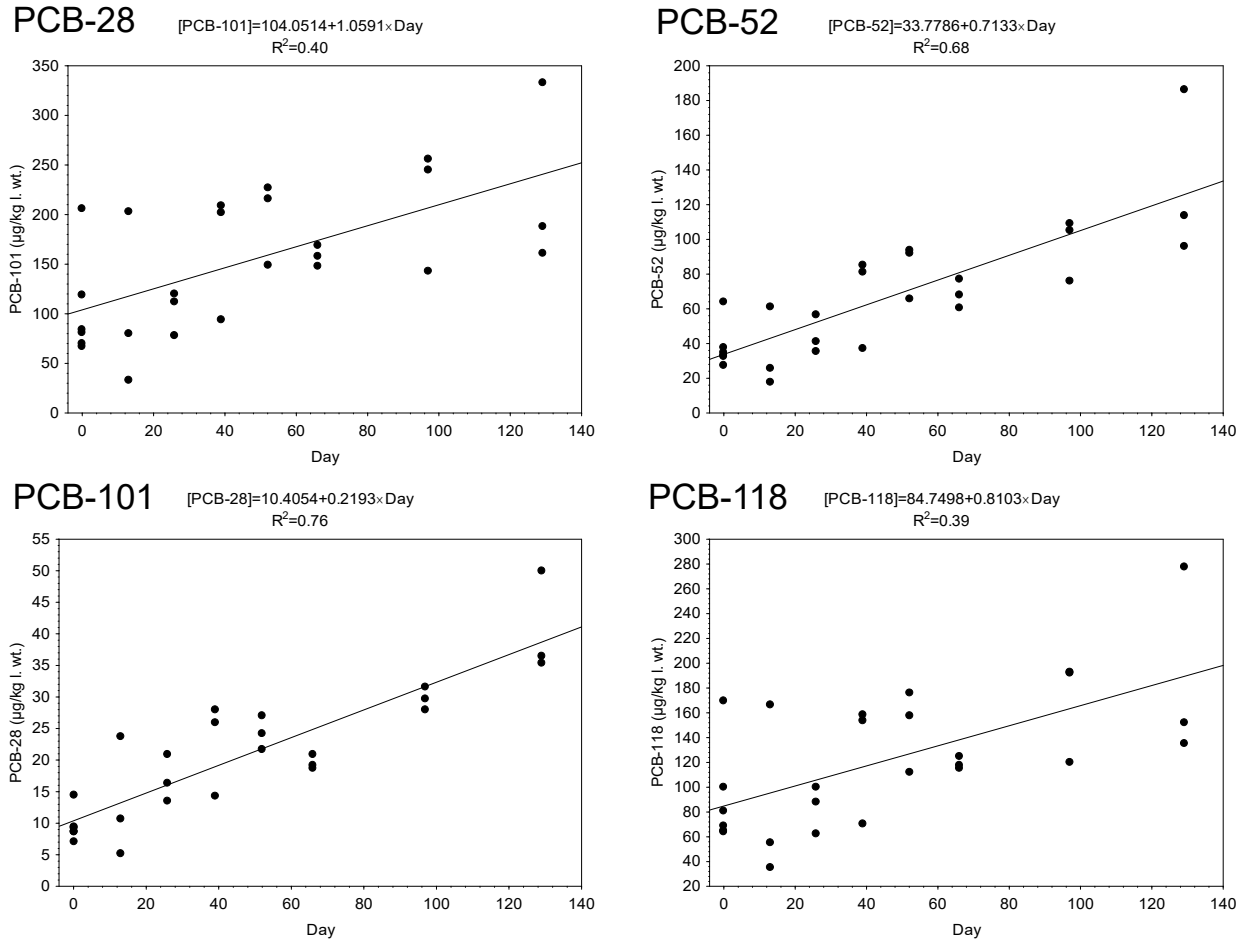

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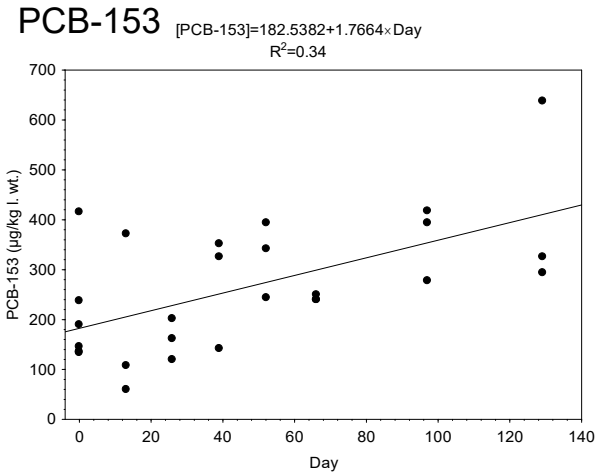
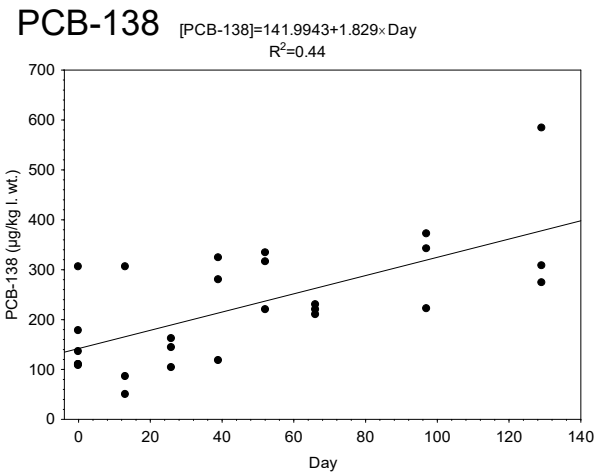
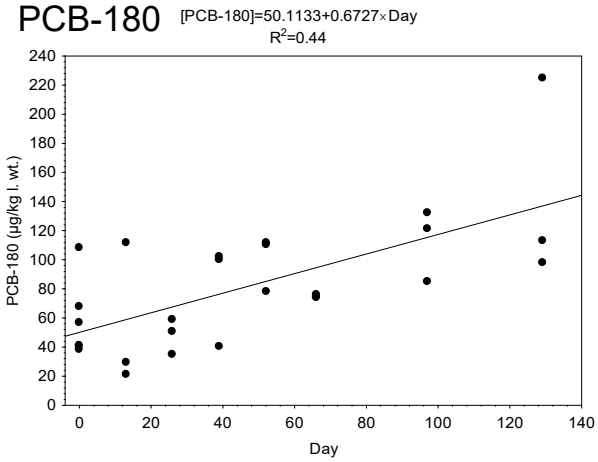
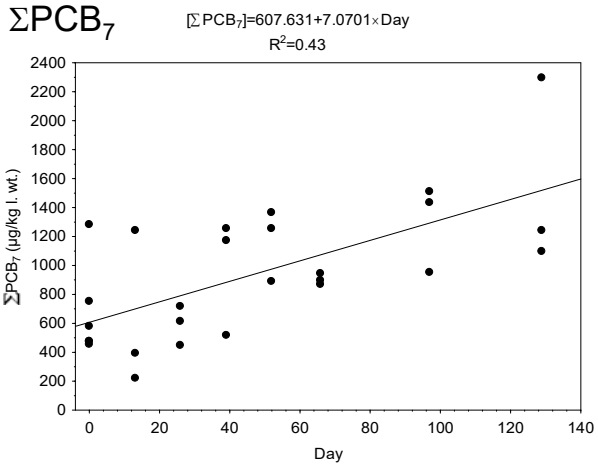
Figure S1 continued









Biota-to-sediment accumulation factor (BSAF)

The biota-to-sediment accumulation factor (BSAF) is calculated as follows:

$BSAF = (C_{Org}/f_{Lip})/(C_{Sed}/f_{OC})$,

where C_{Org} is the wet wt. concentration in the organism, f_{Lip} is the fraction of tissue wet wt. that is lipid, C_{Sed} is the dry wt. concentration in the sediment, and f_{OC} is the fraction of organic carbon in the sediment (g g^{-1} dry wt.). A theoretical prediction of BSAF can be deduced from general equilibration partitioning theory [e.g. 3]:

$$\text{BSAF} = \frac{C_{Lipid}}{C_{OC}}, K_{Lipid} = \frac{C_{Lipid}}{C_W} = K_{OW},\ K_{OC} = \frac{C_{OC}}{C_W} \text{ and } C_{OC} = \frac{C_S}{f_{OC}},$$

where C_{Lipid} is the lipid normalized concentration in the organism, C_{OC} is the organic carbon normalized concentration in the sediment, C_W is the concentration in sediment pore water, C_S is the concentration in sediment (total, dry wt.) and f_{OC} is the fraction of organic content in the sediment (dry:dry).

Since $\text{Log}K_{OC} = \text{Log}K_{OW} - 0.21$ or $K_{OC} = 0.62K_{OW}$ [4], then:

$$\text{BSAF} = \frac{K_{OW} \cdot C_W}{0.62 \cdot K_{OW} \cdot C_W} = 1.6$$

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