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Coplanar and non-coplanar congener-specificity of PCB bioaccumulation and immunotoxicity in sea stars

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Abstract

The sea star *Asterias rubens* (L.), a representative species of the North Sea benthic environment, was exposed to a mixture of 10 selected PCB congeners (3 coplanar or c-PCBs, and 7 non-coplanar) via experimentally contaminated sediments. Both the degree of bioaccumulation and subsequent immunotoxic effects of these PCBs were determined. A strong congener-specificity for both bioaccumulation and immunotoxicity was found as well as a probable induction of a congener-specific detoxification mechanism resulting in the dramatic decrease in body levels of the three coplanar congeners tested (PCBs 77, 126 and 169). Moreover, a correlation was found between the bioaccumulation of c-PCBs and their immunotoxic effects. These findings suggest that coplanar congeners should be included in the list of congeners recommended to be analyzed for biological impact-oriented marine monitoring programmes.

Keywords: Polychlorinated biphenyls; Echinoderms; Bioaccumulation; Immunotoxicity; Detoxification

1. Introduction

Polychlorinated biphenyls (PCBs) are among the persistent organic pollutants (POPs) of greatest concern in marine ecosystems owing to their persistence in the environment and the fact that they are readily bioaccumulated and highly toxic for aquatic organisms (Stebbing et al., 1992; OSPAR, 2000). Although in some cases PCB levels have been shown to decrease regionally, a global decline in PCB concentrations is thought to be unlikely because of continual inputs into the environment mainly due to leakages from landfills and emissions from incinerators (Tanabe, 1988). PCBs have become truly ubiquitous, and have been detected even in the most remote locations, such as the polar regions or the deep sea (Ballschmiter et al., 1997; Stegeman et al., 2001). Another worrisome fact is that the quantities of PCBs still in use in the late 1980s exceeded the amount that had been released into the environment until that time (Ballschmiter et al., 1997; Stegeman et al., 2001). In the marine environment, these contaminants mainly become associated with bottom sediments thereby posing a chronic threat to benthic ecosystems (Fowler et al., 1978; Boese et al., 1996).

The PCB family is composed of 209 different congeners and about half of them have been detected in significant concentrations in environmental samples (Metcalfe, 1994). Until the late 1970s PCBs were mainly analyzed as commercial mixture equivalents (e.g. Aroclor, Kaneclor; Duinker et al., 1991); however, by the late 1980s the need to adopt congener-specific approaches became widely accepted, since it was found that processes such as bioaccumulation, metabolism and toxicity could differ considerably from one congener to another (Duinker et al., 1989).

The International Council for the Exploration of the Sea (ICES) has recommended the systematic consideration of six PCB congeners for monitoring purposes, viz. IUPAC#28, 52, 101, 138, 153 and 180. This selection (with congener #118 added) was subsequently adopted and recommended by the World Health Organization (WHO, 1999). However, it is now widely accepted that the toxicity of such mixtures is mainly due to only a few congeners, viz. the non-*ortho*-substituted and mono-*ortho*-substituted coplanar congeners (Duinker et al.,

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1989; Safe, 1990). These congeners appear to be more problematic than other PCBs; indeed, the former can display coplanar configuration which is very close to that of the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD).

In vertebrates, part of the toxicity of coplanar PCBs (c-PCBs) is known to take place through a receptor-mediated response involving the binding of the contaminant to the cytosolic aryl hydrocarbon receptor (AhR), followed by changes in gene expression (Hahn, 1998; Nebert et al., 2000). Furthermore, c-PCBs are known to induce CYP1A which is the major enzyme responsible for the metabolic activation of promutagens and procarcinogens. However, not all the adverse biological effects of PCBs are attributable to this mechanism; for example, many PCBs can exert toxicity as endocrine disrupters or immunosuppressants through diverse pathways such as alteration of enzymatic activities (kinases and phospholipases), disturbance of Ca^{2+} homeostasis, or modulation of gene expression (Satar, 2000; Arukwe, 2001).

Echinoderms are a major phylum of marine benthic invertebrates which includes a number of species playing key roles in various ecosystems (Menge et al., 1994). Since they are commonly found in coastal and estuarine waters, echinoderms are directly exposed to anthropogenic contaminants which may affect several aspects of their physiology such as reproduction, early development, somatic growth, and neurophysiology (den Besten et al., 1989; Kobayashi, 1995; Coteur et al., 2003). Several authors have proposed the sea star *Asterias rubens* as a suitable sentinel organism (*sensu* Phillips, 1990) for surveying and monitoring marine contamination (Temara et al., 1998, 2002; OSPAR, 2000; Coteur et al., 2003). This top-predator feeds mainly on filter-feeding bivalves and is widely distributed both geographically and bathymetrically in the NE Atlantic and North Sea (Hayward and Ryland, 1990). Furthermore, it is easily identified, collected, and maintained under laboratory conditions, and several experimental and/or field studies have shown that *A. rubens* efficiently bioconcentrates marine contaminants such as metals and PCBs (Sørensen and Bjerregaard, 1991; Rouleau et al., 1993; Temara et al., 1996, 1998; Warnau et al., 1999; den Besten et al., 2001; Coteur et al., 2003; Danis et al., 2003, 2004a). Therefore, the common sea star is considered to be an excellent bioindicator species for monitoring a range of contaminants in the North Sea (Hayward and Ryland, 1990; den Besten et al., 2001; Stronkhorst et al., 2003); however, few studies have investigated in detail PCB bioaccumulation processes in sea stars (Danis et al., 2003, 2004a).

The effects of contaminants on *A. rubens* have focused mainly on its reproductive success (den Besten et al., 1989), early development (Kobayashi, 1995; Coteur et al., 2003), immune system (Coteur et al., 2003; Danis et al., 2004a,b), DNA integrity (Sarkar and Everaarts, 1995) and induction of cytochrome P450 (den Besten et al., 2001; Stronkhorst et al., 2003; Danis et al., 2004a,b). Reactive oxygen species (ROS) production is one of the main immune responses of echinoderms (Sarkar and Everaarts, 1995), and this response is triggered by amoebocytes which are the most active, free-circulating cells found in echinoderm coelomic cavities. Echinoderms lack a specific adaptive immune system and rely on innate responses involving both humoral and cellular components (Chia and Xing, 1996). These processes seem to be very efficient and certainly constitute the main defence against foreign agents. ROS production has recently received much attention in the field of invertebrate immunology and appears to respond to various xenobiotics in echinoderms (Coteur et al., 2001, 2002, 2003; Danis et al., 2004a,b).

Our study focuses on the bioaccumulation and immunotoxicity of key congeners (i.e. those considered as the most abundant or toxic) in the sea star *A. rubens* in order to further assess its value as a bioindicator species for PCBs. Particular care was taken to design experimental conditions to simulate, as closely as possible, natural conditions that are typically encountered in the marine environment (e.g. realistic levels of contaminants and complex mixture exposures).

2. Materials and methods

2.1. Test organisms and sediments

Sea stars (*A. rubens* L.) and sediments (upper 5 cm layer) were collected in Audresselles (Nord-Pas-de-Calais, France) in March 1999 and transported to the IAEA-MEL in Monaco. Prior to experimentation, animals were acclimated to laboratory conditions for 1 month (i.e. constantly aerated, open-circuit 3000 l aquarium; flow rate 300 l h^{-1}; salinity 34 psu; temperature $17 \pm 0.5\,^{\circ}C$; light/dark cycle 12/12 h) during which time they were fed mussels (*Mytilus edulis* L.) *ad libitum*. The sediments were conditioned in a constantly aerated, closed-circuit 400 l aquarium (salinity 34 psu; temperature $4 \pm 0.5\,^{\circ}C$; light/dark cycle 12/12 h), and before use they were characterized for total and organic carbon content and grain-size distribution (sediments were sieved using an Endicots Octagon 200) (Table 1).

2.2. PCB congeners and sediment preparation

Ten PCB congeners were purchased from Promochem GmbH (Germany) as single congeners of certified high purity (from 99.1 to 99.9% purity according to congener). For the experiments a stock solution of each congener was prepared in acetone (1 mg ml^{-1}, Ultrapure grade, Sigma).

Three kilograms of dry sediments were spiked according to the method of Murdoch et al. (1997). The sediments were placed in a 5 l glass bottle containing 500 ml of decanted natural seawater, and an aliquot (~1 μl) of each PCB stock solution was

Table 1
Sediment characteristics: total and organic carbon contents (mg C g^{-1}), and grain-size distribution (%) (mean ± S.D., $n = 6$)

Total carbon (mg g^{-1})	9.29 ± 2.75
Organic carbon (mg g^{-1})	0.27 ± 0.09
Size fractions % (mean ± S.D.)	
500–1000 μm	0.04 ± 0.03
250–500 μm	1.97 ± 0.22
125–250 μm	96.2 ± 0.33
63–125 μm	1.70 ± 0.19
<63 μm	0.08 ± 0.01

then added using a 5 μl Hamilton glass syringe. After 5 min (to allow acetone to evaporate) the sediments were agitated using a rotating plate (150 rpm) for 35 h. At the end of that period, the supernatant seawater was removed and sediments were placed in a 70 l glass aquarium, thoroughly mixed, and maintained for 24 h under flowing seawater (flow rate 15 l h^{-1}, salinity 34 psu; temperature 17 ± 0.5 °C; light/dark cycle 12/12 h) to allow leaching of weakly bound PCBs. The spiked sediments were then allowed to form a continuous layer of approximately 2 cm depth in the experimental aquarium.

2.3. Exposure of sea stars to PCBs

Fifty sea stars of similar size (radius: 59 ± 8 mm) were held for 28 days in the aquarium containing the spiked sediments under the same open-circuit conditions as previously described. Every fourth day the sea stars were allowed to feed overnight on fresh mussels which were supplied (one per sea star) in the evening. The following morning any uningested prey and empty shells were removed. Duration and frequency of feeding were designed in this way in order to minimize any ingestion of PCB-labelled food. At the beginning and end of the experiment, sediments were sampled to check the PCB concentrations. Periodically throughout the experiment, three to four sea stars were collected, rinsed in fresh seawater (particular care was taken to eliminate contaminated sediment particles), rapidly drained of excess water, sampled for their coelomic fluid (see below), and then dissected. Two body compartments (body wall and pyloric caeca) were isolated, weighed (wet weight) and kept frozen (−80 °C) for subsequent analysis of PCB congener concentrations.

2.4. PCB analyses

Extraction of freeze-dried samples, concentration of the extract, removal of lipids, fractionating, regulating gas chromatography conditions, and application of quality assurance/quality control (QA/QC) measures were performed using IAEA-142 reference material (mussel homogenate) according to methods previously described by Villeneuve et al. (1999). QA/QC was carried out using reference material IAEA-142 (mussel homogenate reference material).

2.5. Biokinetic analyses

PCB uptake in the two tissue compartments (body wall and pyloric caeca) was expressed as change in concentration (ng PCB g^{-1} lipid) over time of either single congeners or the sum of the 10 considered congeners. When PCB bioaccumulation tended to reach a steady state during the experiment, the uptake kinetics were best fitted by a single-component, first-order kinetic model:

$$C_t = C_0 + C_{ss}(1 - e^{-kt})$$

where C_0 and C_t are concentrations (ng PCB g^{-1} lipid) at time 0 (background concentration) and t (days), respectively, C_{ss} the incorporated concentration (ng PCB g^{-1} lipid) at steady state, and k is the depuration rate constant (day^{-1}) (Brown et al., 1995). Constants of the uptake equation and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively, using the nonlinear curve-fitting routines in the Systat 5.2.1 software (Wilkinson, 1988). Possible correlations between concentration values for the 10 congeners in the different body compartments were calculated using bivariate correlation procedures in the SPSS 11 software.

2.6. ROS production measurements

At days 0, 2, 7, 16, and 28 during the contamination period, coelomic fluid was collected from four sea stars for amoebocyte ROS production analysis according to the method described by Coteur et al. (2002). An aliquot of 3 ml of coelomic fluid, obtained by cutting the tip of the longest arm of the sea stars, was poured into 3 ml anticoagulant buffer (1.2×10^{-2} M EDTA in Ca-, Mg-free artificial seawater, CMFASW; Noble, 1970) at 4 °C. The amoebocyte concentration of this suspension was determined using a Thoma haemocytometer. The suspension was then centrifuged (400 × g for 10 min, 4 °C) and the supernatant replaced by CMFASW to obtain a final concentration of $1 \pm 0.25 \times 10^6$ cells ml^{-1}. This concentration was double-checked as described above.

ROS were measured using peroxidase/luminol-enhanced chemiluminescence (PLCL), using a EG&G LB-9507 luminometer (Coteur et al., 2002). Measurements were normalised with the actual amoebocyte concentration in each sample and expressed as the sum of all 10 min-interval measurements for 10^6 cells ("total chemiluminescence"). Possible correlations between ROS production and PCB concentrations in sea star tissues were then tested using bivariate correlation routines in the SPSS 11 software.

3. Results

3.1. PCB congener bioaccumulation

The uptake kinetics of the 10 PCB congeners in two different body compartments (body wall and pyloric caeca) are shown in Fig. 1 (sum of 10 PCBs, $\sum_{10}$PCBs) and Figs. 2 and 3 (individual congeners). The monitoring of congener concentrations in sediments after spiking and after 28 days of exposure (Table 2) showed that $\sum_{10}$PCBs concentration decreased from 178 ng g^{-1} dry weight at the beginning of the experiment to 129 ng g^{-1} dry weight at day 28. Concentrations decreased with similar rates regardless of the congener, indicating that no particular process other than desorption from sediments was responsible for this loss. Assuming the concentration decrease rate was roughly constant, this would result in a time-integrated, mean sediment concentration of approximately 154 ng g^{-1} dry weight.

When considering the sum of the 10 PCB congeners, the uptake in both body compartments displayed saturation kinetics (Fig. 1, Tables 3 and 4). The concentrations measured after 28 days of exposure were 15 ± 1.69 μg g^{-1} lipids in the body wall and 6.83 ± 2.81 μg g^{-1} lipids in the pyloric caeca. Correspond-

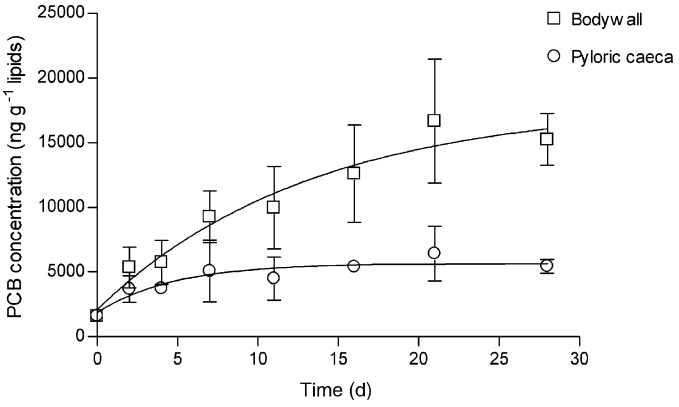

Fig. 1. Uptake kinetics of the sum of 10 PCB congeners (mean concentration in ng g^{-1} total lipids, $n = 3$) in the body wall and the pyloric caeca of sea stars exposed to spiked sediments.

ing mean estimated steady-state concentrations (i.e. $C_0 + C_{ss}$) were 18.1 and 6.06 μg g^{-1} lipids, respectively, suggesting that during the time course of the experiment both body compartments reached saturation.

When individual PCB congeners were considered, uptake kinetics in body wall and pyloric caeca displayed saturation kinetics for all congeners except the coplanar ones (Fig. 2, Tables 3 and 4). In the case of coplanar congeners (PCB 77 and 126), uptake kinetics displayed a fairly unpredictable bioaccumulative behaviour. Indeed, their concentration increased at the beginning of the experiment (until days 7–11), and then dropped sharply. PCB 169 displayed very low accumulation in either

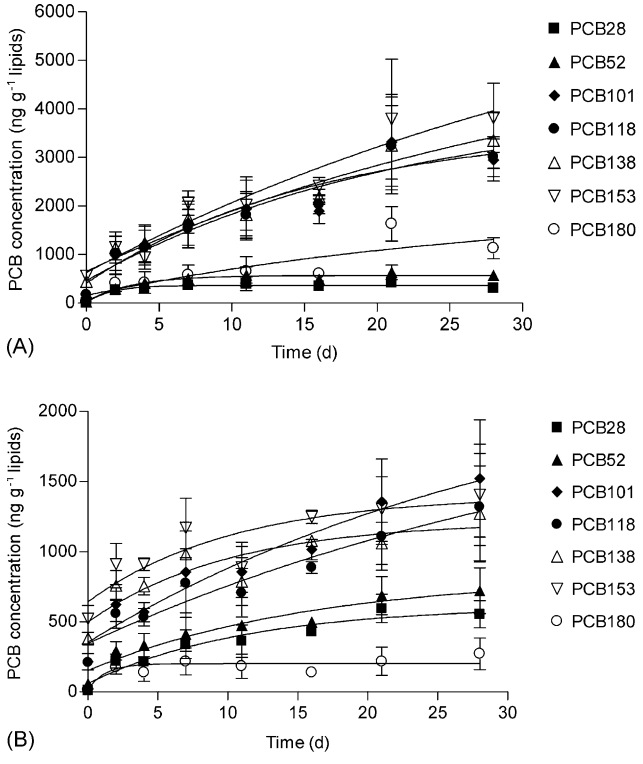

Fig. 2. Individual uptake kinetics of seven non-coplanar PCB congeners (mean concentration in ng g^{-1} total lipids, $n = 3$) in (A) the body wall and (B) the pyloric caeca of sea stars exposed to spiked sediments.

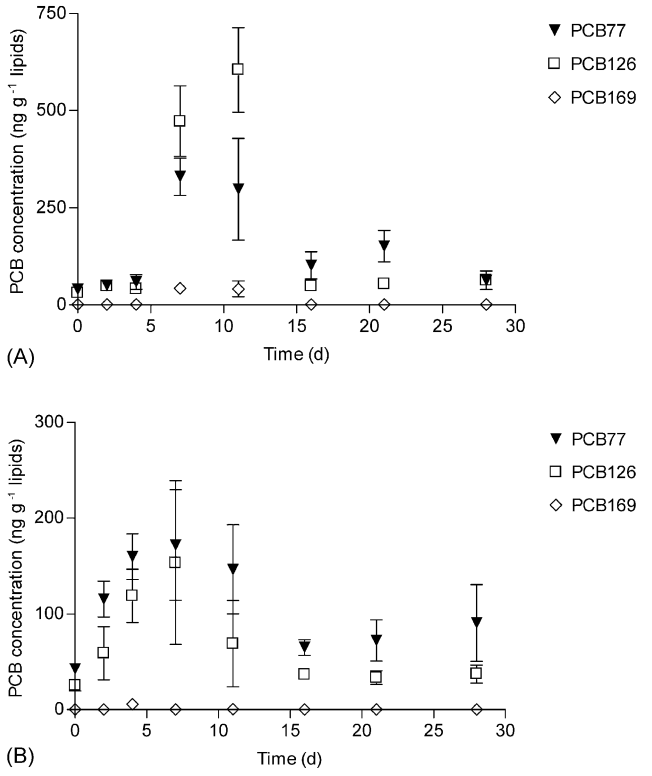

Fig. 3. Individual uptake kinetics of three c-PCB congeners (mean concentration in ng g^{-1} total lipids ± S.D., $n = 3$) in (A) the body wall and (B) the pyloric caeca of sea stars exposed to spiked sediments.

compartment. Regarding the non-coplanar congeners the estimated steady-state concentrations ($C_0 + C_{ss}$) in the body wall ranged from 367 ng g^{-1} lipids (PCB 28) to 6.3 μg g^{-1} lipids (PCB 153) (ratio = 1:17), whereas in the pyloric caeca, values ranged from 590 ng g^{-1} lipids (PCB 28) to 1.7 μg g^{-1} lipids (PCB 101) (ratio = 1:3). Except in the case of PCB 180, C_{ss} was found to be related to the K_{OW} of considered congeners in the body wall, while this was not the case when considering the pyloric caeca.

In order to verify a possible metabolic transformation of the c-PCBs, ratios of the non-coplanar PCB 153 (which is not metab-

Table 2
Concentration of the different PCB congeners in the experimental sediments (mean concentration ± S.D.; ng g^{-1} dry weight; $n = 3$, except before addition, $n = 1$)

	Before PCB addition	After PCB addition and 24 h under flowing seawater	After 28 days of experiment
PCB 28	0.044	5.9 ± 1.0	4.2 ± 0.8
PCB 52	0.004	7.1 ± 1.1	5.1 ± 1.0
PCB 77	0.003	5.2 ± 1.0	3.5 ± 0.9
PCB 101	0.008	26 ± 4.0	18 ± 3.7
PCB 118	0.005	27 ± 4.5	19 ± 4.2
PCB 126	<0.002	13 ± 2.0	9.0 ± 1.9
PCB 138	0.007	30 ± 4.0	22 ± 3.8
PCB 153	0.007	30 ± 4.0	22 ± 4.2
PCB 169	<0.002	3.0 ± 0.2	2.4 ± 0.2
PCB 180	<0.002	31 ± 5.0	24 ± 4.8
$\sum_{10}$PCBs	0.084	178 ± 27	129 ± 26

Table 3
Background PCB concentrations (ng g^{-1} lipids) in *Asterias rubens* and parameters and statistics of the equations describing the uptake kinetics of the $\sum_{10}$PCBs and individual non-coplanar congeners in two body compartments of *A. rubens* exposed to the PCBs via sediments[a]

Congener	C_{BKD}	Uptake kinetic parameters			R^2_{corr}
		C_0 (ASE)	C_{ss} (ASE)	k (ASE)	
Body wall					
PCB 28	8.33	–	367 (21.2)	0.33 (0.08)	0.69
PCB 52	31.1	–	593 (29.5)	0.22 (0.04)	0.83
PCB 101	200	191 (241)	3000 (426)	0.08 (0.03)	0.81
PCB 118	278	195 (287)	3530 (695)	0.06 (0.03)	0.76
PCB 138	989	629 (209)	4340 (1940)	0.03 (0.02)	0.82
PCB 153	1330	685 (261)	5580 (3750)	0.03 (0.03)	0.78
PCB 180	32.2	75.1 (134)	1520 (536)	0.05 (0.04)	0.72
$\sum_{10}$PCBs	2980	1510 (1300)	16600 (2910)	0.07 (0.03)	0.78
Pyloric caeca					
PCB 28	14.4	5.69 (68.3)	584 (89.1)	0.10 (0.05)	0.71
PCB 52	48.1	55.8 (84.2)	670 (116)	0.10 (0.05)	0.69
PCB 101	203	236 (183)	1470 (624)	0.06 (0.05)	0.58
PCB 118	214	204 (168)	1210 (498)	0.06 (0.06)	0.56
PCB 138	540	475 (163)	758 (243)	0.09 (0.09)	0.44
PCB 153	743	505 (211)	852 (221)	0.12 (0.11)	0.42
PCB 180	27.8	–	200 (31.5)	0.41 (0.32)	0.18
$\sum_{10}$PCBs	1890	1830 (941)	5230 (1390)	0.09 (0.08)	0.52

ASE: asymptotic standard error; R^2_{corr}: corrected determination coefficient; C_{BKD}: background PCB concentrations measured in sea stars before starting the experiment.

[a] Model used: $C(t) = C_0 + C_{ss}(1 - e^{-kt})$, where $C(t)$ and C_0 are PCB concentrations (ng g^{-1} lipids), respectively, at time t (days) and at time 0, and C_{ss} is the PCB concentration incorporated at steady state; k depuration rate constant (day^{-1}).

olized; Atuma et al., 1996) to PCB 77, PCB 126 and PCB 169 have been computed for sediments and sea star tissues at different times during the experiment (Fig. 4). The ratio did not vary much for sediments, suggesting that no significant metabolization of these congeners occurred. In contrast, the ratios of PCB 153 to c-PCBs increased up to a factor 30,000 in sea star tissues (Fig. 4).

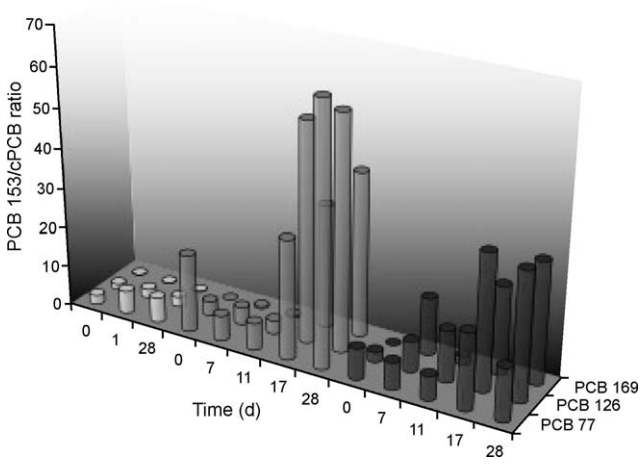

Fig. 4. Variation of the ratio between PCB 153 and c-PCBs 77, 126 and 169 in sediments (white), body wall (light grey) and pyloric caeca (dark grey) at different times during the exposure period. Time 0: background ratios (before spiking). To fit the figure scale, PCB 153:169 ratio is divided by a factor 100 in body wall, and by 1000 in pyloric caeca.

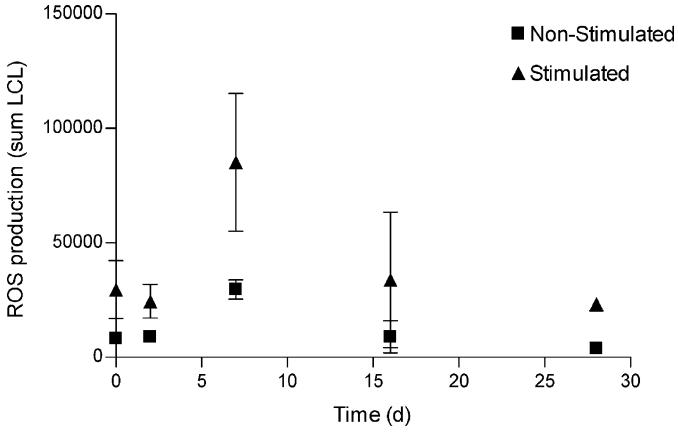

Fig. 5. ROS production (mean total chemiluminescence, RLU ± S.E., $n = 3$) by nonstimulated (squares) or bacteria-stimulated (dots) amoebocytes in sea stars exposed to spiked sediments.

Correlations between incorporated concentrations of different congeners were also examined, and the bioaccumulation of several congeners was found to be highly correlated in both tissues. The strongest correlations were found for PCBs 28 versus 52 ($r \geq 0.91$), 101 versus 118 ($r \geq 0.99$), and 138 versus 153 ($r \geq 0.98$). Significant correlations were also found between c-PCB concentrations in body wall and pyloric caeca, both for intra- and inter-tissue comparisons. In particular, PCB 77 and PCB 126 concentrations were always closely correlated, sometimes with very high correlation coefficients (e.g., $r = 0.95$, $p \leq 0.0001$ in body wall).

3.2. Effects of PCB congeners on ROS production

In order to gain some insight into the effects of PCB congeners in this species, reactive oxygen species (ROS) production was analysed for non-stimulated and bacteria-stimulated amoebocytes. During the experiment, the variation of ROS production displayed a distinct behaviour whereby ROS production in both non-stimulated and bacteria-stimulated amoebocytes increased sharply during the first week of exposure, then declined and reached a constant level, close to background levels, during the last two weeks (Fig. 5). Most notably, the kinetic behaviour of ROS production (Fig. 5) matched perfectly that displayed by c-PCB accumulation pattern in sea star tissues (Fig. 3). Correlation analysis performed between ROS production and c-PCB tissue concentrations demonstrated strong correlations between c-PCB concentrations in body wall and ROS production by non-stimulated amoebocytes, with correlation coefficients ranging from 0.82 to 0.87 (Table 5).

4. Discussion

The present study is a complement to two previous studies dealing with single PCB congener accumulation in sea stars, in the form of two structurally contrasting radiolabelled PCB con-

Table 4
PCB concentrations (mean ± S.D.; ng g^{-1} lipids; $n = 3$) over time (days) in the body wall (A) and pyloric caeca (B) of the sea star *Asterias rubens* exposed to spike sediments

Time	PCB 28	PCB 52	PCB 77	PCB 101	PCB 118	PCB 126	PCB 138	PCB 153	PCB 169	PCB 180	$\sum_{10}$PCBs
(A) Body wall											
0	7.63 ± 1.21	25.5 ± 3.54	39.5 ± 3.54	112 ± 2.12	171 ± 1.41	30.7 ± 6.66	444 ± 38.4	559 ± 17.1	0.90 ± 0.12	28.0 ± 1.41	1600 ± 290
2	265 ± 66.8	292 ± 74.6	49.7 ± 18.2	987 ± 384	1020 ± 352	48.3 ± 10.7	1140 ± 246	1150 ± 322	0.85 ± 0.24	411 ± 170	5360 ± 1560
4	302 ± 84.5	411 ± 64.5	59.0 ± 33.0	1205 ± 377	1140 ± 359	41.3 ± 4.93	1230 ± 379	935 ± 240	0.76 ± 0.14	419 ± 222	5750 ± 1690
7	374 ± 72.9	501 ± 95.8	330 ± 83.3	1650 ± 453	1540 ± 397	473 ± 157	1740 ± 301	2060 ± 251	42.7 ± 24.8	578 ± 206	9280 ± 2000
11	398 ± 139	579 ± 167	298 ± 227	1950 ± 649	1820 ± 484	605 ± 154	1820 ± 436	2020 ± 517	41.2 ± 35.2	653 ± 301	9990 ± 3180
16	371 ± 61.6	531 ± 96.2	94.5 ± 51.3	2230 ± 714	2390 ± 744	51.5 ± 8.85	2570 ± 771	2820 ± 830	0.92 ± 0.11	765 ± 328	11800 ± 3470
21	423 ± 84.2	637 ± 148	151 ± 70.7	3320 ± 985	3240 ± 829	53.7 ± 8.50	3250 ± 1000	3790 ± 1240	0.74 ± 0.06	1630 ± 356	16700 ± 4800
28	318 ± 37.7	584 ± 50.3	57.5 ± 35.7	2905 ± 366	2960 ± 359	63.8 ± 7.59	3290 ± 484	3720 ± 613	0.91 ± 0.20	1180 ± 207	15100 ± 1690
(B) Pyloric caeca											
0	12.7 ± 2.08	61.5 ± 14.9	42.7 ± 7.02	215 ± 99.1	209 ± 65.8	25.3 ± 10.2	382 ± 77.2	522 ± 167	0.07 ± 0.02	16.3 ± 4.16	162 ± 442
2	221 ± 87.5	294 ± 113	116 ± 32.35	624 ± 209	560 ± 179	59.0 ± 48.1	759 ± 184	913 ± 254	0.07 ± 0.02	177 ± 85.6	3700 ± 1020
4	214 ± 35.4	334 ± 118	160 ± 33.94	569 ± 97.6	532 ± 88.4	119 ± 39.6	756 ± 88.4	910 ± 63.6	5.71 ± 9.78	139 ± 89.1	3730 ± 350
7	340 ± 177	411 ± 208	172 ± 99.86	855 ± 504	776 ± 424	154 ± 148	983 ± 394	1170 ± 366	0.08 ± 0.01	218 ± 168	5080 ± 2380
11	362 ± 201	477 ± 63.7	147 ± 80.35	856 ± 314	707 ± 301	69.0 ± 78.4	788 ± 296	890 ± 311	0.44 ± 0.66	184 ± 149	4480 ± 1660
16	429 ± 26.7	499 ± 35.0	65.0 ± 14.00	1016 ± 126	887 ± 71.9	36.7 ± 3.21	1080 ± 55.0	1250 ± 80.8	0.06 ± 0.01	139 ± 31.4	5400 ± 351
21	595 ± 174	689 ± 235	72.3 ± 37.29	1360 ± 527	1110 ± 415	33.3 ± 12.7	1060 ± 259	1300 ± 398	0.06 ± 0.01	218 ± 175	6440 ± 2130
28	539 ± 141	688 ± 241	104 ± 62.34	1410 ± 630	1230 ± 570	42.5 ± 16.8	1220 ± 493	1370 ± 519	0.05 ± 0.01	227 ± 183	6830 ± 2810

Table 5
Correlation coefficients and associated Bonferronni probabilities (in brackets) between ROS production in non-stimulated (NS) or stimulated (S) amoebocytes and coplanar PCB concentrations determined in body wall (A) or pyloric caeca (B)

	PCB #77	PCB #126	PCB #169
(A) Body wall			
NS	0.865 (0.005)	0.822 (0.023)	0.838 (0.014)
S	0.662 (ns)	0.707 (ns)	0.707 (ns)
(B) Pyloric caeca			
NS	0.531 (ns)	0.664 (ns)	0.559 (ns)
S	0.447 (ns)	0.605 (ns)	0.43(ns)

geners, PCB 153 and c-PCB 77 (Danis et al., 2003, 2004a,b, respectively). The previous experiments, along with those performed here, were designed to obtain complementary information on PCB bioaccumulation in the sea star *A. rubens* using different techniques (GC-ECD versus radiotracer techniques), uptake routes (seawater, food, sediments), and contaminants (10 different congeners), but always using contaminant exposure concentrations that were of the same order of magnitude as those that can occur in the marine environment.

Results of the present study clearly demonstrated that all PCB congeners tested (except coplanar PCB 169) were readily accumulated in sea star tissues, with transfer factors from sediments of 120 in the body wall and 40 in the pyloric caeca. These values, although slightly lower, are comparable to those found for sediment exposure in previous studies using the single congener PCB 153 (Danis et al., 2003); this suggests that the presence of a mixture of congeners does not interfere with uptake efficiency of PCB 153 in sea star tissues (Table 6).

When considering the sum of the 10 PCB congeners tested ($\sum_{10}$PCBs), uptake kinetics in body wall and pyloric caeca were best described by continuous increasing functions eventually reaching a steady-state in their bioaccumulation. Between the two tissues, body wall was found to be the most effective accumulator, which is in agreement with previous observations (Danis et al., 2003). In addition, body wall was the only compartment in which a relation was found between the K_{OW} of the considered congeners (except PCB 180) and their saturation concentration. Because it is an efficient accumulator, easily dissected, and constitutes ca. 75% of the total sea star body weight, the body wall could serve as an ideal target tissue for biomonitoring studies using sea stars. Thus body wall analysis of PCBs

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would complement the information gathered through analyses of pyloric caeca which are often the only tissues examined in field studies (e.g. den Besten et al., 2001; Stronkhorst et al., 2003).

When examining individual congeners separately, all the non-coplanar PCBs followed the same saturation kinetics as $\sum_{10}$PCBs, although they attained different steady-state concentration values. Surprisingly, the uptake kinetics for coplanar congeners (except PCB 169), displayed totally different patterns; the concentrations of these PCBs reached a peak value after 7–11 days of exposure, according to the c-congener, and then suddenly dropped to initial values. This behaviour could be due either to a change in bioaccumulation parameters (decrease in uptake rate or increase in loss rate) or, alternatively, to the progressive activation of an efficient detoxification mechanism that triggers metabolization of these c-PCBs specifically. In view of existing literature, the second hypothesis appears to be the most plausible. Indeed, specific induction of P450 enzymatic activity has been reported in echinoderms exposed to c-PCBs (e.g. Danis et al., 2004a,b; den Besten et al., 1993). In addition, the fact that ratios of the non-*ortho* substituted coplanar congeners (PCBs 77, 126 and 169) to the non-coplanar PCB 153 increased during the experiment support the existence of a metabolic transformation of these c-PCB congeners.

c-PCBs have close structural similarities with polychlorodibenzo-*p*-dioxins (PCDDs) (Metcalfe, 1994; Walker and Peterson, 1994). Those c-PCBs with vicinal hydrogen atoms in *o*, *m* positions and 0-1 *ortho* chlorine atoms, such as PCBs 77 and 126, are generally thought to be targets of cytochrome P450 (CYP) isozymes and consequently are metabolized (Hong et al., 1998). Regarding the effects of PCB 77, caution should be exercised since existing evaluations of the toxicity (and metabolism) are hampered by variations in toxic effects and in different species (Safe, 1994). Whereas the CYP enzyme system and its inducibility have been extensively described in vertebrates, much less is known for invertebrates despite the fact that the CYP1A system has been reported in four invertebrate phyla: annelids, arthropods, echinoderms and molluscs (Lee, 1981; Bucheli and Fent, 1995). In particular, Snyder (1998) has recently identified the first echinoderm CYP genes in digestive tissues of a sea urchin (*Lytechinus anamesis*), and evidence for the presence of P450 enzymes belonging to the CYP1, CYP2, and CYP3 subfamilies has been reported previously in sea stars (den Besten et al., 1993; Danis et al., 2004a,b).

Chlorine substitution degree and pattern in PCB 77 and PCB 126 are generally thought to be adequate for a possible metabolization; however those of PCB 169, given the fact that this congener is highly chlorinated, are not so ammenable to breakdown of the biphenyl structure (Walker and Peterson, 1994). Nevertheless our observations indicate that a metabolization of all the c-PCBs we considered seems to occur, and that PCBs 77, 126 and 169 would undergo similar metabolic processes in sea stars. Enzymes from the cytochrome P450 superfamily could play a pivotal role in these processes.

Most noteworthy, the immune function (ROS production) that was measured during the bioaccumulation experiment followed exactly the same pattern as c-PCB uptake; i.e., after an initial increase to day 7, ROS production fell to control levels from day 16 until the end of the experiment. This observation could be due to toxicity and subsequent impairment in the function of amoebocytes occurring when PCB concentrations reach a certain level within the organism's tissues. However, non-coplanar PCB congeners (viz., those showing increasing concentrations over time) were seen to have no effect on ROS production in echinoderms (Coteur et al., 2001; Danis et al., 2004a). In contrast, c-PCBs are well documented to specifically stimulate ROS production by amoebocytes in echinoderms (Coteur et al., 2001; Danis et al., 2004a,b) as well as in other marine organisms (Wilbrink et al., 1991; Duffy et al., 2002). Therefore, the return of ROS production to normal levels is clearly related to the decrease in c-PCB concentrations in the sea stars. This is also suggested by the strong correlation found between c-PCB concentrations and ROS production values. Such a drop in ROS production was also reported in *A. rubens* during experimental exposure to the single c-PCB 77 congener (Danis et al., 2004a). The involvement of a congener-specific protective mechanism through the cytochrome P450 system was also suggested, since a clear induction of a CYP1A immunopositive protein (CYP1A IPP) was measured following c-PCB 77 and 126 exposure, but not following exposure to PCB 153 (Danis et al., 2004a,b).

The immunotoxic effects measured in the present study show the potential importance of ROS production as a biomarker of c-PCB exposure, and constitute a good basis to argue for the usefulness of echinoderms as bioindicator organisms for PCBs. In addition, this immune function is of high ecological relevance, since it provides direct information about the health of the organisms. Indeed, high levels of ROS production can lead to the production of large quantities of reduction products, such as the hydroxyl radical ($OH^{\bullet}$) or the superoxide anion ($^{\bullet}O_2^-$) which are extremely potent oxidants that react with cellular macromolecules (Chia and Xing, 1996).

It is interesting to note that while the shape of the ROS production kinetics closely reflected the observations of Danis et al. (2004a), the pattern of the PCB 77 uptake kinetics observed here did not. In the present study PCB 77 was bioaccumulated in *A. rubens* tissues following bell-shaped kinetics, whereas Danis et al. (2004a) described a continuous increasing uptake finally reaching a steady state in concentration. In view of the above discussion, this kinetic discrepancy is most certainly due to the different analytical methods used in both studies. The present study used classical chemical analysis (GC-ECD) which unequivocally identifies each congener, whereas Danis et al. (2004a) used liquid scintillation techniques to measure the ^{14}C activity of radiolabelled PCB 77. Although the use of a radiotracer has numerous advantages in studying c-PCB biokinetics (e.g. increase in detection sensitivity), the major drawback of this technique is that it detects only ^{14}C and not specifically the PCB congeners. Hence, no differentiation can be made between the congener itself and possible degradation products if the labelled molecule is metabolized. Therefore, based on the observations noted in our study as well as those reported by Danis et al. (2004a,b) on ROS production and CYP1A IPP induction, we suspect that the saturation kinetics reported for radiolabelled PCB 77 bioaccumulation resulted from detection of both ^{14}C-

PCB 77 and its potential ^{14}C-metabolite(s) rather than being due to a different kinetic behaviour of the congener in the two studies.

In general, the findings from this study demonstrate the existence of a relationship between the bioaccumulation of coplanar PCB and resultant immunotoxic effects. Furthermore, these results have provided evidence for congener-specific toxicity under complex, realistic conditions (i.e. exposure to a mixture of PCB congeners bound to sediments containing ecologically relevant PCB concentrations). These findings underscore the need to provide information in natural environments about similar coplanar-specific biological effects in other organisms, especially the "classical" bioindicator species such as bivalves. The recommendations for PCB monitoring that are presently adopted by international organisations (e.g. EU, ICES, WHO) and widely followed in the scientific community generally address only a limited set of non-coplanar PCB congeners (viz. #28, 52, 101, 118, 138, 153 and 180). The latter ones are well known to be the most relevant in terms of PCB abundance in biota and environment (OSPAR, 2000), but not at all with respect to their toxicity and as a threat to marine ecosystems. Our results show that coplanar congeners were alone responsible for the observed immunotoxicity, despite the fact that they were much less abundant in the experimental environment (as occurs in the natural environment) and far less bioconcentrated than the congeners "recommended" for monitoring studies. Because PCBs affect a pivotal function of the immune system (ROS production) of sea stars in a congener-specific way, coplanar PCBs may represent a potential threat to echinoderm populations, and hence to benthic communities in general. Therefore, we propose that coplanar congeners be included, as far as possible, on the list of congeners to be monitored in studies dealing with biological impact-focused assessments of pollution in the marine environment.

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