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Bioaccumulation of PCBs in the sea urchin *Paracentrotus lividus*: seawater and food exposures to a ^{14}C-radiolabelled congener (PCB#153)

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The sea urchin Paracentrotus lividus is a valuable indicator for PCB contamination.

Abstract

Adult *Paracentrotus lividus* were exposed to a ^{14}C-labelled PCB congener (PCB#153) using two different exposure modes: (1) the surrounding sea water and (2) the food (viz. the phanerogam *Posidonia oceanica* and the brown alga *Taonia atomaria*). Uptake kinetics from water and loss kinetics after single feeding were followed in four body compartments of the sea urchins (body wall, spines, gut and gonads). Results indicate that PCB bioaccumulation in *P. lividus* varies from one body compartment to another, with the exposure mode and the nature of the food. The echinoids accumulate PCB#153 more efficiently when exposed via water than via the food (the transfer efficiency is higher by one order of magnitude). Target body compartments of PCB#153 were found to be body wall and spines when individuals were exposed via water, and gut when they were exposed via food. It is concluded that *P. lividus* is an efficient bioaccumulator of PCB and that it could be considered as an interesting indicator for monitoring PCB contamination in the marine environment.
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Keywords: PCB; Bioaccumulation; Echinoderm; *Paracentrotus lividus*

1. Introduction

Polychlorinated biphenyls (PCBs) are hydrophobic contaminants that have been shown to cause various adverse effects on a wide variety of living species (see e.g. Harding and Addison, 1986). Because of their toxic potential, their production and use have been strictly regulated and then banned in many countries since the mid-1970s (Metcalfe, 1994). However, due to their extreme resistance to physicochemical and biological degradation, PCBs have become widely spread in the environment, and particularly in the marine environment.

Information on PCB bioaccumulation in marine benthic organisms is scarce and is generally limited to experiments using laboratory-contaminated sediments as a source of contamination (Meador et al., 1995; Weisberg et al., 1996; Boese et al., 1997). In addition, few studies have taken into account essential species, upon which depends the ecosystem structure and/or functioning (Fowler and Oregioni, 1976; Philips, 1976).

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The echinoid *Paracentrotus lividus* qualifies as an excellent bioindicator species of PCB contamination in the Mediterranean Sea. It is indeed a widely distributed, sedentary and abundant species that plays key roles in various Mediterranean ecosystems (Hayward and Ryland, 1990), including seagrass meadows where usual indicators such as mussels are poorly represented. Its value as a bioindicator species for metal contamination is well documented by numerous laboratory and field studies (e.g. Warnau et al., 1995, 1996a, 1996b, 1998). Several studies have investigated the toxicological effects of PCBs on sea urchin early development (e.g. Trieff et al., 1988; Kobayashi, 1995; Weisberg et al., 1997; Schweitzer et al., 1997, 2000) or immune system (Coteur et al., 2001), but virtually nothing is known about PCB bioaccumulation kinetics in echinoderms in general and in *P. lividus* in particular.

The aim of this study was to further assess the value of *P. lividus* as an indicator species of environmental PCB contamination. Therefore, PCB bioaccumulation was investigated in *P. lividus* exposed through its two main contamination pathways: sea water and food.

Congener IUPAC#153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) was selected as representative of PCBs for this study: it is the most abundant congener in marine biota (Stebbing et al., 1992) and has been shown to be an good indicator congener in PCB monitoring programmes (e.g., Metcalfe, 1994; Atuma et al., 1996). Finally, in order to study environmentally realistic simulated contaminant levels, the selected PCB congener was ^{14}C-labelled and measured using a sensitive radiodetection technique (liquid scintillation).

2. Materials and methods

2.1. Sampling

The echinoid *Paracentrotus lividus* (Lamarck), the phanerogam *Posidonia oceanica* (L.) and the brown alga *Taonia atomaria* were collected in June 1999 by SCUBA diving between 5 and 10 m depth in a *P. oceanica* meadow off "la Pointe des Douaniers" (Cap d'Ail, France). Prior to experimentation, specimens were acclimated to laboratory conditions for 1 month (constantly aerated open circuit aquaria, salinity 36‰, 17 ± 0.5 °C, 12/12 h dark/light cycle).

2.2. Radiotracer

^{14}C-labelled 2,2′,4,4′,5,5′-hexachlorobiphenyl (purity >95%) was purchased from Sigma Chemicals, USA. Specific activity was 25 mCi mmol^{-1}. Stock solutions were prepared in acetone at a concentration of 1 μg ml^{-1}.

2.3. Sample treatment and liquid scintillation counting

Water samples (2 ml) were directly transferred to 20 ml glass scintillation vials (Packard, USA) and to 10 ml of Ultima Gold XR® (Packard Instruments) scintillation liquid were added. Subsamples of vegetal and echinoid tissues were placed in a vial containing 2 ml of Acetonitrile® in an ultrasonic bath for 10 min. Acetonitrile® was then collected and replaced by another 2 ml of Acetonitrile® and the ultrasonic operation was repeated for a further 10 min. This treatment gave 4 ml of liquid phase (the extraction) and a residue. The residue was digested overnight at 70 °C with 2 ml of Soluene®, and 10 ml of Hionic Fluor® scintillation liquid were then added. The liquid phase (4 ml) was added to 16 ml of filtered sea water and extracted twice using 2 ml of n-hexane (Sigma, USA) under constant agitation. The organic phase (4 ml) and the aqueous phase (20 ml) were treated separately. The entire organic phase and 2 ml of the aqueous phase were each added separately to 10 ml of Ultima Gold XR® scintillation liquid.

^{14}C-radioactivity was measured using a 1600 TR Liquid Scintillation Analyzer (Packard), compared to standards of known activities, and corrected for quenching, background and physical decay of the tracer. Counting times were adjusted to obtain counting rates with relative errors lower than 5%. PCB concentrations were eventually expressed on a total lipid content basis. Lipids were determined according to the method of Barnes and Blackstock (1973).

2.4. Experimental procedure

2.4.1. Sea water exposure

Ten sea urchins (diameter 50 ± 7 mm) were placed for 9 days in a 20 l glass aquarium (constantly aerated closed circuit aquarium, salinity 36‰, 17 ± 0.5 °C, 12/12 h dark/light cycle) containing natural sea water spiked with ^{14}C-labelled PCB#153. One day before starting the experiment, two 5 l glass beakers were filled with filtered sea water (36‰, 17 ± 0.5 °C), spiked with the radiolabelled PCB, and constantly stirred for 24 h using an orbital agitation plate. Contaminated water was then poured into the glass aquarium and uncontaminated sea water was added to obtain a final volume of 20 l. Sea water and radiotracer were renewed daily throughout the experiment. Activity was checked before and after each renewal to assess the stability of the labelled PCB concentration in sea water (Table 1). The echinoids were fed unlabelled fresh *Posidonia oceanica* leaves every second day, just before the water renewal. After 2 h, uningested leaves were removed in order to avoid as much as possible PCB incorporation via the food. At different times (0, 2, 5 and 8 days) echinoids ($n=3$) were collected, dissected into four body compartments (body

Table 1
Characteristics of the background and added concentrations of PCB#153[a]

	Compartment	PCB#153 concentration
Background	Sea water	0.026 ng l^{-1} ($n=6$)
Added	Sea water (dissolved + particulate)	31.4 ± 15.6 ng l^{-1} ($n=36$)
	P. oceanica	5.5 ± 0.5 ng g^{-1} wet wt ($n=12$)
	T. atomaria	17.9 ± 5.6 ng g^{-1} wet wt ($n=12$)

[a] Background concentrations were measured in sea water the day before starting the experiments; added concentrations were measured in samples of sea water, *Posidonia oceanica* shoots, and *Taonia atomaria* thallia regularly collected in the experimental microcosms throughout the experiment.

wall, spines, gut, gonads), and radioanalyzed to determine the body distribution of the PCB.

2.4.2. Food exposure

Shoots of *Posidonia oceanica* and thallia of *Taonia atomaria* were exposed for 14 days in two separate glass aquaria containing 10 l natural sea water spiked with ^{14}C-labelled PCB#153 (Table 1). Sea water and tracer were renewed daily. Two groups of 20 sea urchins (diameter 52 ± 6 mm) were placed in two 20 l polyvinylchloride aquaria (constantly aerated open circuit, salinity 36‰, 17 ± 0.5 °C, 12/12 h dark/light cycle) and allowed to feed overnight on the previously contaminated food. In parallel, two groups of three echinoids were placed in separate parts in both aquaria, and fed with uncontaminated food to serve as a control for possible cross-contamination through sea water. After an 18 h feeding period, the remaining labelled food was removed and echinoids were fed ad libitum with uncontaminated *P. oceanica* or *T. atomaria* until the end of the experiment. Faeces were removed twice a day in order to avoid as much as possible any cross contamination via PCB leaching from the faeces. At different times (days 2, 4, 9, 11, 14, and 17 after feeding), individuals ($n=3$) were dissected into three body compartments (body wall, gut, gonads) in order to determine loss kinetics and body distribution of ingested PCB.

2.5. Data analyses

Uptake of PCB#153 from sea water was expressed as change in PCB concentration (ng g^{-1} total lipids) over time. Uptake kinetics were described by using the following exponential model (eq. (1)):

$$C(t) = A\, e^{kt} \quad (1)$$

where $C(t)$ is the PCB concentration taken up at time t (days) (ng g^{-1} total lipids) and k is the rate constant (day^{-1}).

Regarding elimination of the radiotracer ingested with food, loss kinetics were best described using a simple exponential model (eq. (2)):

$$C(t) = C(0)\, e^{-kt} \quad (2)$$

where $C(0)$ and $C(t)$ are the ^{14}C-labelled PCB#153 concentrations (ng g^{-1} total lipids) at time 0 (beginning of the loss experiment) and at time t (days), and k is the rate constant (day^{-1}).

Constants of the model and their statistics were calculated by iterative adjustment and Hessian matrix computation, respectively, using the nonlinear curve-fitting routines in the Systat® 5.2.1 software (Wilkinson, 1988).

Differences between PCB#153 concentrations in the different body compartments of the echinoid were tested by one-way ANOVA and the multiple comparison test of Tukey (Zar, 1996). Changes in PCB distribution among body compartments were tested for significance using the G-test (adapted from the log-likelihood ratio test) for $2\times k$ contingency tables (Zar, 1996). Prior to this test, data were arcsin-transformed, using the correction of Freeman–Tukey (1950) as described in Zar (1996). The level of significance for statistical tests was always set at $\alpha=0.05$.

3. Results

3.1. Sea water experiment

The uptake of PCB#153 by *P. lividus* exposed to contaminated sea water was followed in four body compartments (body wall, spines, gut and gonads) (Fig. 1). Parameters and statistics of the uptake kinetics are given in Table 2. Estimated uptake rate constants were quite homogeneous among the different body compartments. This indicates that bioaccumulation efficiency was similar, particularly in body wall and spines and in gut and gonads.

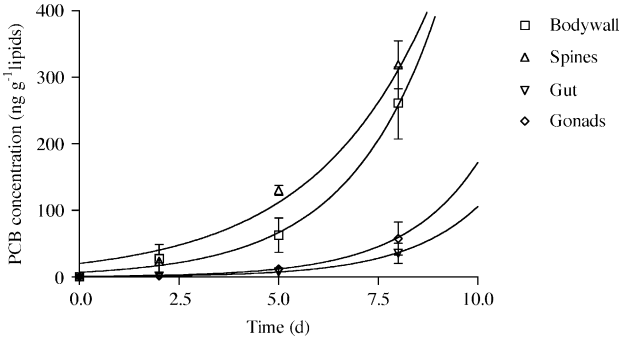

Fig. 1. Uptake kinetics of ^{14}C-labelled PCB#153 from seawater in four body compartments of the sea urchin (mean concentration ng g^{-1} total lipids ± SD, $n=3$).

Table 2
Parameters and statistics of the equation fitting the uptake of ^{14}C-labelled PCB#153 in the body compartments of echinoids exposed via sea water[a]

	A (ASE)	k (ASE)	R^2
Body wall	7.05 (4.8)	0.45 (0.09)	0.93
Spines	20.5 (5.2)	0.34 (0.03)	0.97
Gut	0.53 (0.88)	0.53 (0.21)	0.80
Gonads	0.86 (1.4)	0.53 (0.21)	0.80

[a] $C(t)=Ae^{kt}$. $C(t)$, ^{14}C-labelled PCB#153 concentration (ng g^{-1} lipids) at time t (days); A, ordinate at the origin (ng g^{-1} lipids); k, rate constant (day^{-1}); ASE, asymptotic standard error; R^2, determination coefficient.

Table 3 presents the concentrations of stable PCB#153 corresponding to the radiolabelled congener incorporated at different times over the exposure period (Table 3a) and the corresponding distribution among the considered body compartments (Table 3b). The body wall and spines concentrated ^{14}C-labelled PCB#153 to the highest degree, reaching mean concentrations up to 262 and 319 ng g^{-1} lipids, respectively. These concentrations were one order of magnitude higher than in gut and gonads ($p_{\text{Tukey test}}<0.0001$; Table 3a). Body distribution of the ^{14}C-labelled PCB#153 varied significantly (G-test; $p<0.05$) among sampling days (Table 3b). However, spines and, secondarily, body wall always displayed the highest proportion (29–62%) of total body load of incorporated PCB.

3.2. Food experiment

Two sets of sea urchins were allowed to feed overnight either on *P. oceanica* or on *T. atomaria* previously exposed to ^{14}C-labelled PCB#153. Sea urchins were then placed in uncontaminated conditions in order to determine the loss kinetics of the ingested PCB. Analysis of control animals showed that there was no significant cross contamination through sea water due to food leaching.

Table 3
Concentrations and distribution of PCB#153 incorporated in the different body compartments of the echinoids exposed to the congener via sea water[a,b]

	Body wall	Spines	Gut	Gonads
A.				
Day 2	27.6 ± 20.8[a]	23.5 ± 4.480[a]	1.43 ± 1.71[a]	1.56 ± 2.39[a]
Day 5	62.7 ± 25.8[b]	130 ± 7.450[c]	7.35 ± 3.16[d]	12.0 ± 5.15[d]
Day 8	262 ± 54.3[e]	319 ± 35.90[e]	35.4 ± 15.3[f]	57.6 ± 24.9[f]
B.				
Day 2	46.3 ± 23.3	50.5 ± 26.3	1.8 ± 2.5	2.0 ± 2.9
Day 5	28.7 ± 7.8	62.3 ± 8.1	8.7 ± 2.7	5.7 ± 2.1
Day 8	38.6 ± 5.2	47.3 ± 1.8	9.9 ± 4.6	9.2 ± 4.8

[a] A. PCB#153 concentrations (mean ng g^{-1} lipids ± SD, $n=3$). Mean concentrations sharing the same superscript do not differ significantly between each other.

[b] B. PCB#153 distribution (mean % ± SD, $n=3$) among body compartments.

Table 4
PCB concentrations (ng g^{-1} lipids; mean ± SD, $n=3$) measured in the sea urchin body compartments after a single feeding, using two different food (*Posidonia oceanica* vs *Taonia atomaria*)[a]

	Body wall	Gut	Gonads
P. oceanica			
Day 2	10.0 ± 1.74[a]	10.8[a] ± 2.03[a]	2.26 ± 0.064[a]
Day 4	11.8 ± 2.59[c]	20.8[c] ± 4.47[c]	25.9 ± 8.79[c]
Day 9	4.58 ± 0.69[e]	4.60[e] ± 1.95[e]	3.28 ± 0.96[e]
Day 11	5.51 ± 2.07[g]	3.87 ± 0.52[g]	3.31 ± 0.81[g]
Day 14	2.37 ± 1.08[h]	0.095 ± 0.106[h]	1.00 ± 0.64[h]
Day 17	1.83 ± 1.40[i]	0.16 ± 0.057[i]	0.59 ± 0.13[i]
T. atomaria			
Day 2	19.6 ± 6.22[j]	5.94 ± 0.26[j]	2.75 ± 0.23[j]
Day 4	12.7 ± 2.02[k]	7.90 ± 1.35[k]	2.59 ± 1.73[k]
Day 9	4.86 ± 1.96[l]	6.13 ± 0.90[l]	1.65 ± 0.23[l]
Day 11	3.01 ± 1.82[m]	5.74 ± 1.14[m]	1.14 ± 0.071[m]
Day 14	3.26 ± 1.30[o]	1.57 ± 0.35[o]	1.31 ± 0.26[o]
Day 17	0.77 ± 0.43[p]	1.09 ± 0.26[p]	1.12 ± 0.47[p]

[a] Mean concentrations sharing the same superscript do not differ significantly among each other ($p_{\text{Tukey test}}>0.05$).

A latency time of 2–4 days was observed before the contaminant concentration reached a maximum in the body compartments (Table 4). Loss kinetics were calculated taking into account the period between that maximal value and the end of the experiment.

Loss kinetics were similar for both foods considered (*P. oceanica* and *T. atomaria*) (Figs. 2 and 3). The main differences between the two feedings was the decrease of radioactivity in the gut contents: after *P. oceanica* feeding, ^{14}C-labelled PCB activity decreased exponentially in the gut contents, whereas, with *T. atomaria*, it decreased linearly as a function of time (data not shown).

Loss of ingested ^{14}C-labelled PCB#153 followed a one-component exponential model in each body compartments (Table 5). The kinetics were characterized

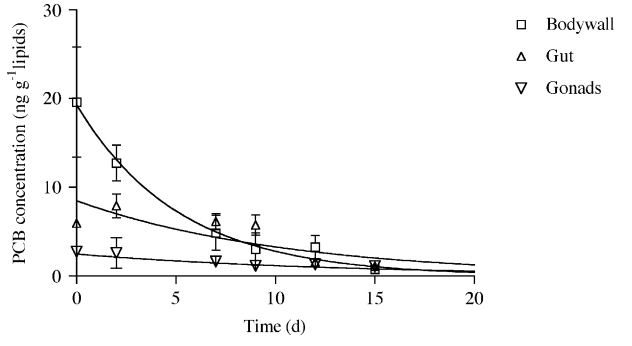

Fig. 2. Loss kinetics of ^{14}C-labelled PCB#153 (mean concentration ng g^{-1} total lipids ± SD, $n=3$) in three body compartments of the sea urchin after a single feeding on radiolabelled food (*Posidonia oceanica*).

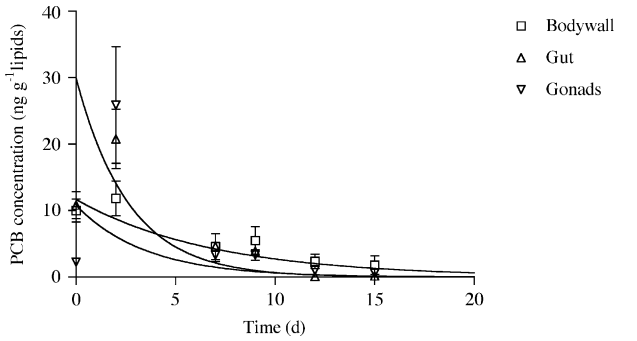

Fig. 3. Loss of ^{14}C-labelled PCB#153 (mean concentration ng g^{-1} total lipids ± SD, $n=3$) in three body compartments and in the gut content after a single feeding on radiolabelled food (*Taonia atomaria*).

by rapid loss of ingested PCB: calculated biological half-lives ($Tb_{1/2}$) ranged between 2.5 and 9.2 days.

4. Discussion

The present study reports the first experimental data on the bioaccumulation kinetics of a key PCB congener in the sea urchin *Paracentrotus lividus,*a common species widely distributed in the Mediterranean Sea and on the NE Atlantic coast.

The biokinetic experiments carried out in this study were performed using a ^{14}C-labelled PCB congener (2,2′,4,4′,5,5′-hexachlorobiphenyl) and were designed in order to expose sea urchins to moderate to high PCB concentrations as commonly found in the marine environment.

PCB#153 was shown to be efficiently accumulated from sea water by the sea urchin. This observation matches other uptake experiments using for example polychaetes and fish exposed to Aroclor (Fowler et al., 1978; Shaw and Connell, 1987). All the considered body compartments (body wall, spines, gut, gonads) accumulated the congener following exponential uptake kinetics. Even if the exposure period was rather short (8 days), concentration factors (ratio between ^{14}C-labelled PCB in body compartments and in surrounding sea water) were quite elevated and ranged between 10^3 (in the soft tissues: gut and gonads) and 10^5 (in the calcified tissues: body wall and spines). This indicates the efficiency of the sea urchin organs as bioaccumulator compartments and, hence, their usefulness as tools for the survey and biomonitoring of PCB contamination in the marine environment. Being easily dissected and constituting ca. 90% of the total body weight, body wall and spines are of particular interest with respect to field studies, and they should be recommended as body compartments to monitor.

Table 5
Parameters and statistics of the equation describing the loss of PCB#153 from the sea urchin body compartments after a single feeding on *Posidonia oceanica* and *Taonia atomaria*[a]

	$C(0)$ (ASE)	k (ASE)	R^2	$Tb_{1/2}$
P. oceanica				
Body wall	11.7 (0.99)	0.145 (0.023)	0.83	4.8
Gut	20.8 (1.30)	0.282 (0.042)	0.94	2.5
Gonads	29.9 (1.14)	0.376 (0.049)	0.98	1.8
T. atomaria				
Body wall	19.3 (1.43)	0.193 (0.031)	0.87	3.6
Gut	8.5 (0.84)	0.095 (0.019)	0.71	7.3
Gonads	2.49 (0.46)	0.075 (0.031)	0.42	9.2

[a] Equation is $C(t)=C(0)e^{-kt}$; where $C(t)$ and $C(0)$ are ^{14}C-labelled PCB#153 concentrations (ng g^{-1} lipids) at time t (days) and time 0, respectively, and k is the rate constant (day^{-1}). ASE, asymptotic standard error; R^2, corrected determination coefficient; $Tb_{1/2}$, biological half-life (days).

After feeding with common food of radiolabelled, loss of PCB#153 displayed exponential kinetics. The loss was quite rapid in each compartment. Biological half-lives of the PCB congener ranged between 2.5 and 9 days. This indicates a low retention of the PCB taken up through the trophic chain. Nevertheless, over the long term, this pathway could contribute significantly to the total load of PCB in the sea urchin. It is notewothy that, even if PCB#153 was three times more concentrated in *T. atomaria* thallia given as food than in *P. oceanica* leaves (see Table 1), the congener concentration incorporated in the sea urchin soft tissues (gut and gonads) were higher when the animals were exposed via *P. oceanica* (see Tables 4 and 5). This would indicate a higher bioavailability of the PCB congener when it is incorporated in the *P. oceanica* tissues than in those of *T. atomaria*. Conversely, retention of ingested PCB was 3–5-fold stronger in soft tissues of sea urchins fed *T. atomaria* (see Table 5).

While this work constitutes the first report on PCB bioaccumulation kinetics in an adult sea urchin, previous studies have used ^{14}C-radiolabelled PCB to examine bioaccumulation in other aquatic organisms (e.g. Goerke and Ernst, 1977; Gooch and Hamdy, 1982; Schweitzer et al., 1997). However, these studies are few and mostly concern PCBs as Aroclor mixtures (see e.g. Butcher et al., 1997).

5. Conclusions

The main advantage of the ^{14}C approach to measure PCB bioaccumulation in aquatic biota is obviously the high sensitivity and the rapidity of the detection, compared to analytical techniques using gas chromatography. Furthermore, it allows working with low, realistic PCB concentrations, and assessing uptake in individual organs which are often too small to be analyzed by classical chemical without pooling.

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