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Bioaccumulation of PCBs in the cuttlefish *Sepia officinalis* from seawater, sediment and food pathways

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Bioaccumulation of PCBs by cuttlefish is studied, via seawater, sediments and their food.

Abstract

The cuttlefish *Sepia officinalis* was selected as a model cephalopod to study PCB bioaccumulation via seawater, sediments and food. Newly hatched, juvenile cuttlefish were exposed for 17 days to environmentally realistic concentrations of ^{14}C-labeled 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB#153) (18 ng PCB l^{-1} seawater; 30 ng PCB g^{-1} dry wt sediments; *Artemia salina* exposed to 18 ng PCB l^{-1} seawater). Accumulation of PCB#153 was followed in three body compartments: digestive gland, cuttlebone and the combined remaining tissues. Results showed that (1) uptake kinetics were source- and body compartment-dependent, (2) for each body compartment, the accumulation was far greater when *S. officinalis* was exposed via seawater, (3) the cuttlebone accumulated little of the contaminant regardless of the source, and (4) the PCB congener showed a similar distribution pattern among the different body compartments following exposure to contaminated seawater, sediment or food with the lowest concentrations in the cuttlebone and the highest in the remaining tissues. The use of radiotracer techniques allowed delineating PCB kinetics in small whole organisms as well as in their separate tissues. The results underscore the enhanced ability of cephalopods to concentrate organic pollutants such as PCBs, and raise the question of potential risk to their predators in contaminated areas.
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1. Introduction

Among persistent organic pollutants (POPs), poly-chlorobiphenyls (PCBs) are a class of anthropogenic contaminants of long-standing environmental concern (Livingstone et al., 2000). Represented by 209 congeners, PCBs are widespread, highly conservative and are readily accumulated in living organisms. PCBs entering marine waters become available for marine organisms whose biology can be affected at various organizational levels (Metcalfe, 1994). Moreover, these contaminants may also be biomagnified in food webs raising a potential risk for high trophic level predators. Marine invertebrates take up these contaminants via three main routes: ambient seawater (through gills and body surfaces), direct contact with sediments, and ingestion of food. Previous studies using sea stars and sea urchins (Danis et al., 2003, in press) have shown that the efficiency of PCBs bioaccumulation depends on the source of contamination (viz. seawater is probably the main contamination source) and on the considered congener (Danis and Warnau, in preparation).

Cephalopods, including benthic (octopus), necto-benthic (cuttlefish), neritic and oceanic (squids) species, are widely distributed in the world oceans. They generally display short life span and high growth rates. Owing to the economic value of many cephalopod species, they are of high commercial interest for fisheries (Forsythe and Heukelem, 1987; Navarro and Villanueva, 2000). Moreover, cephalopods play key roles in marine ecosystems, being both active predators of fish and crustaceans (Castro and Guerra, 1990; Rodhouse and Nigmatullin, 1996) and important prey items for marine mammals, seabirds and fish (Clarke, 1996; Croxall and Prince, 1996; Klages, 1996; Smale, 1996). Therefore, cephalopods can be considered as an important vector for transferring potentially hazardous contaminants to top marine predators (Bustamante et al., 1998; Weisbrod et al., 2000, 2001).

Cephalopods are known to accumulate numerous contaminants among which are POPs such as organo-chlorine pesticides or PCBs (Tanabe et al., 1984; Kawano et al., 1986; Yamada et al., 1997; Weisbrod et al., 2000, 2001; Ueno et al., 2003). However, very little is known about bioaccumulation capacity depending upon the routes of exposure to these contaminants; such data are needed to further assess the potential impact of organic pollutants on cephalopod populations. For these reasons, experiments were designed to study the bioaccumulation of PCBs by the common cuttlefish *Sepia officinalis* following exposure via seawater, sediments and food.

PCB biokinetics were determined using radiotracer techniques in order to measure fluxes at environmentally realistic concentrations (Danis et al., 2003, in press). These techniques have already proven useful when examining kinetic behaviour in organs that are too small for employing classical PCB analytical measurements. The PCB congener #153 (2,2′,4,4′,5,5′ hexa-chlorobiphenyl) was selected as a model PCB, since it is the most abundant in marine biota and has been recognized as an indicator of total PCB contamination (see e.g., Stebbing et al., 1992; Atuma et al., 1996).

2. Materials and methods

2.1. Organisms

Seven- to nine-day old newly hatched cuttlefish (hereafter called juveniles) were used in the experiments. Cuttlefish eggs were spawned in the laboratory by adults collected by net fishing off Monaco and were maintained in aquaria until hatching. Young cuttlefish were kept in a separate aquarium (open circuit, 20 l h^{-1} flow rate, constant aeration, 36 p.s.u., 16.5 ± 0.5 °C, 12 h/12 h

light/dark cycle) and fed brine shrimp (*Artemia salina*) until used in the experiments.

2.2. Radiotracer

The PCB radiotracer (^{14}C-labelled 2,2′,4,4′,5,5′-hexachlorobiphenyl; purity $\geq 95\%$) was purchased from Sigma Chemicals, USA. Specific activity was 925 MBq mmol^{-1} and stock solutions were prepared in acetone at a concentration of 1 μg ml^{-1} and stored at -20 °C until used.

2.3. Liquid scintillation counting

Water samples (2 ml) containing the radiotracer were directly transferred to 20 ml glass scintillation vials (Packard, USA) and mixed with 10 ml of scintillation liquid (Ultima Gold XR®, Packard, USA). Sediment and biota samples (cuttlefish tissues and brine shrimp) were ultrasonified twice for 10 min, each time with 2 ml of Acetonitrile® (Packard, USA). This treatment produced a liquid phase (4 ml) containing the extracted ^{14}C-labelled PCB and a residue. The residue was digested overnight at 70 °C with 2 ml of Soluene® (Packard, USA) and mixed with 10 ml of scintillation liquid (Hionic Fluor®, Packard, USA). The liquid phase was added to 16 ml of filtered seawater and extracted twice using 2 ml of n-hexane (Sigma, USA) under agitation. The organic phase (4 ml) and the aqueous phase (20 ml) were treated separately. The whole organic phase, and 2 ml of the aqueous phase were mixed separately with 10 ml of Ultima Gold XR® scintillation liquid. The treatment is summarized in Fig. 1.

β-Emission of the tracer was measured in the samples using a 1600 TR Liquid Scintillation Analyzer (Packard). The counting time was selected to obtain counting rates with relative propagated errors less than 5% (maximum counting duration 2 h). Radioactivity measured in the samples was compared to standards and corrected for quenching, background and physical decay of the tracer. PCB concentrations were expressed on a total lipid content basis where lipids were determined according to the method of Barnes and Blackstock (1973).

3. Experimental procedures

3.1. Uptake from seawater

Juvenile cuttlefish (mean wet wt $\pm$ SD 0.141 ± 0.039 g; $n = 25$) were placed for 20 d in a 70 l glass aquarium containing natural seawater spiked with 18 ng ^{14}C-PCB#153 l^{-1} seawater. This concentration corresponds to a moderate level of contamination in the North Sea (Stebbing et al., 1992). One day before starting the
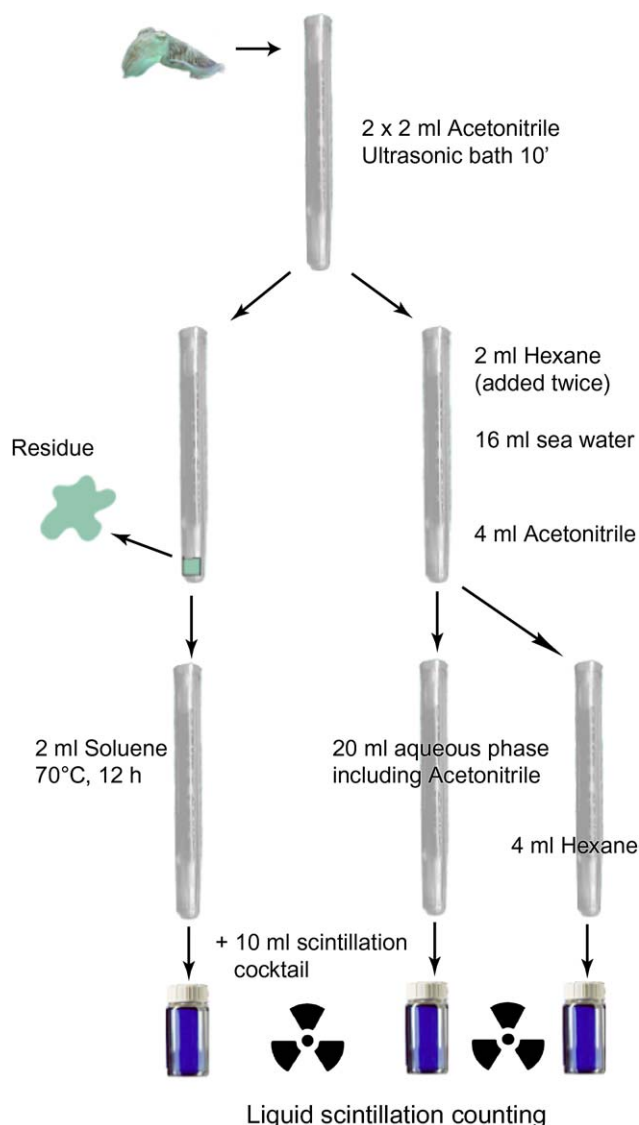
Fig. 1. Diagrammatic representation of sample processing prior to liquid scintillation counting.

termine the distribution of the radiotracer among digestive gland, cuttlebone and the remaining tissues.

3.2. Uptake from sediments

Sediments (2.5 kg dry wt) from the North Sea (Audresselles, Pas-de-Calais, France) were contaminated for 4 d with the ^{14}C-labeled PCB using the rolling jar method (Murdoch et al., 1997). Sediments were then placed in a 70 l glass aquarium and maintained overnight under flowing seawater (open circuit, 20 l h^{-1} flow rate, 36 p.s.u., 16.5 ± 0.5 °C) to allow any loosely bound contaminant to leach. The seawater level in the aquarium was then reduced so that a 2 cm layer of natural seawater was running over the 3 cm layer of spiked sediments. This was done in order to minimize cuttlefish movements required for feeding and to optimize their contact time with sediments. Juvenile cuttlefish (mean wet wt $\pm$ D 0.124 ± 0.046 g; $n = 25$) were then placed for 17 d in the aquarium during which time the sediment and seawater radioactivity was periodically checked. These measurements showed that ^{14}C-PCB radioactivity and concentration (9.49 ± 1.14 ng g^{-1} dry wt) remained constant in the sediments throughout the experiment, and that no radioactivity could be detected in seawater. Cuttlefish were fed twice daily with *Artemia salina* and any uningested food was removed after 1 h and β-counted. No activity could be detected in the brine shrimp. Cuttlefish were periodically sampled to follow PCB uptake kinetics and distribution among the tissues over time.

3.3. Uptake from food

Before the feeding experiment, brine shrimp were exposed for 7 d in a glass aquarium containing 4 l of filtered seawater spiked with 18 ng ^{14}C-PCB#153 l^{-1} (24-h water spiking procedure as described above for seawater). Radiolabelled seawater was renewed daily and brine shrimp were regularly fed a mixture of phytoplankton. After 7 d, brine shrimp were used to feed the juvenile cuttlefish. (Fresh brine shrimp were labelled daily to have 7-d labelled food available each day throughout the experiment.) Juvenile cuttlefish (mean wet wt $\pm$ SD 0.130 ± 0.029 g; $n = 25$) were placed in individual plastic containers (10 cm diameter, 5 cm height) and held in a 70 l glass aquarium (open circuit, 20 l h^{-1} flow rate, 36 p.s.u., 16.5 ± 0.5 °C). During the entire experiment (17 d), individuals were allowed to ingest radiolabelled *A. salina* twice a day for 1 h; food was given in excess and any non-ingested shrimp remaining after the feeding period were immediately removed). Cuttlefish were periodically sampled over time to follow PCB uptake kinetics and distribution among the tissues.

experiment, four 5 l glass beakers were filled with filtered seawater (36 p.s.u., 16.5 ± 0.5 °C), spiked with the PCB stock solution, and constantly stirred using an orbital agitation plate. Contaminated seawater was poured into the glass aquarium which was subsequently filled to a final volume of 70 l with uncontaminated seawater. This operation (spiked seawater preparation) was performed daily throughout the duration of the experiment and radiolabelled seawater was renewed daily in the aquarium. Radioactivity was checked before and after each seawater renewal to assess the stability of the labelled PCB concentration. All cuttlefish were fed twice a day with *Artemia salina* and were periodically sampled. After 1 h, uningested brine shrimp were removed to limit as much as possible incorporation of PCB through the food. At each sampling time, three individuals were dissected to de-

3.4. Data analyses

Uptake of the ^{14}C-PCB congener from seawater, sediments and food was expressed as change in PCB concentration (ng g^{-1} total lipids) over time. Radiotracer uptake kinetics were described either by using a linear model (Eq. (1)), a saturation exponential model (Eq. (2)), or a combined model (logistic plus exponential) (Eq. (3)):

$$C(t) = C_0 + kt \tag{1}$$

$$C(t) = C_{ss}(1 - e^{-kt}) \tag{2}$$

$$C(t) = \frac{C_{ss}(1 - e^{-kt})}{1 + e^{-k(t-I)}} \tag{3}$$

where $C(t)$, C_0, and C_{ss} are the ^{14}C-PCB concentrations (ng g^{-1} total lipids), respectively, at time t (d), at time 0, and at steady-state, k is the rate constant (d^{-1}) and I is the time (d) at the inflexion point. The model showing the most accurate fit (based on the calculation of the determination coefficient, R^2, and examination of the residuals) was used. Constants and statistics of the different models were estimated by iterative adjustment of the models and Hessian matrix computation, respectively, using the nonlinear curve-fitting routines in Systat® 5.2.1 (Wilkinson, 1988). Differences among ^{14}C-PCB concentrations in the different body compartments were tested using one-way ANOVA and the multiple comparison test of Tukey (Zar, 1996). After arcsine-transformation of data (using the correction of Freeman–Tukey, 1950; in Zar, 1996), changes in ^{14}C-PCB body distribution were tested for significance using the G-test (adapted from the log-likelihood ratio test) for $2 \times k$ contingency tables (Zar, 1996). The level of significance for statistical tests was always set at $\alpha = 0.05$.

4. Results

Uptake kinetics of ^{14}C-PCB#153 by *S. officinalis* were determined by exposing juveniles via three different pathways: seawater, sediments and food. Accumulation was followed in three body compartments: digestive gland, cuttlebone and remaining tissues (which included all the other tissues and organs). Accumulation was also considered in whole-body organisms using reconstituted data of the separate tissues.

4.1. Uptake from seawater

Bioaccumulation kinetics and their parameters in the different body compartments of the cuttlefish following exposure to environmentally realistic seawater PCB concentrations (18 ng l^{-1}) are shown in Fig. 2 and Table 1. Except for cuttlebone, uptake of the contaminant was best described using linear models. Cuttlebone took up little, if any, PCB congener. Among the compartments, the remaining tissues concentrated PCB#153 to the greatest degree, almost one order of magnitude higher than in the digestive gland and two orders of magnitude higher than in the cuttlebone ($p_{Tukey\ test} \leq 0.0001$. Concentration factors (ratio between PCB concentration in organism and in an equal weight of seawater) were calculated for the compartments considered and for the whole body (Table 2).

The tissue distribution of ^{14}C-PCB#153 varied significantly (log-likelihood ratio, G-test) throughout the experiment (Fig. 3). At the beginning of the experiment, all of the contaminant (100%) was found in the remaining tissues. Then, during the first days of exposure, a progressive transfer to the digestive gland took place, reaching a peak of about 15% of the total load after 4 d. The remaining tissues always contained the major fraction (80–100%) of the total ^{14}C-PCB body load, whereas very low proportions were found in the cuttlebone (0–2.5%).

4.2. Uptake from sediments

As observed in the seawater experiment, cuttlebone did not appear to accumulate ^{14}C-PCB during sediment exposure. Accumulation kinetics in the digestive gland were best described by a combined model (logistic + exponential components), whereas accumulation in the remaining tissues and in whole organisms was best fitted by a saturation exponential model (Fig. 4, Table 1). Similar to the seawater experiment, the body compartment that took up PCB#153 to the greatest degree was the remainder. Nevertheless, transfer factors between sediments and organisms remained low, reaching maximum values of 5.3 (Table 2).

The tissue distribution of ^{14}C-PCB was determined at different times during the experiment (Fig. 5). The remaining tissues contained the major fraction (84–99%) of the total PCB body load, whereas the lowest proportions were found in the cuttlebone (0–1.3%). At day 1, the remaining tissues contained 99% of the radiotracer, with a progressive transfer to the digestive gland taking place during the first days of exposure and reaching a peak of about 13% of the total load after 4 d.

4.3. Uptake from food

Throughout the experiment, juvenile cuttlefish were fed radiolabelled brine shrimp ad libitum for 1 h, twice a day. Uptake kinetics of ^{14}C-PCB#153 ingested with food followed the combined (logistic + exponential) model in the digestive gland and the linear model in
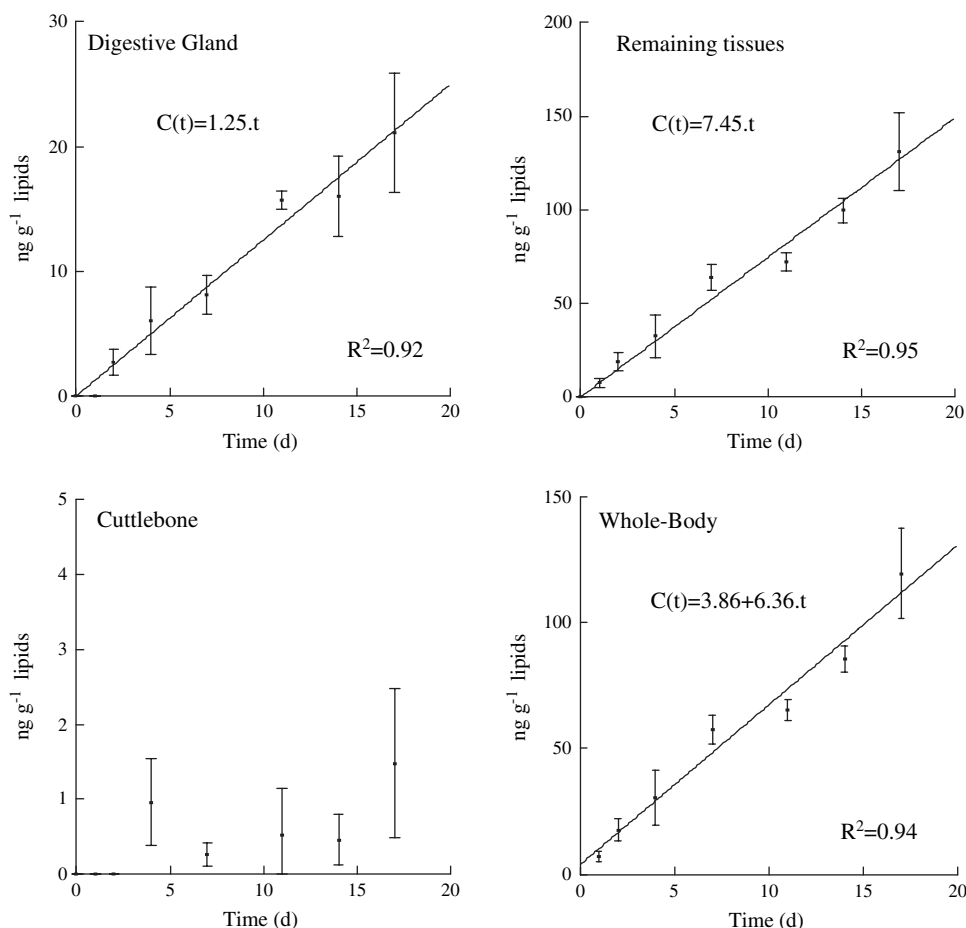
Fig. 2. *Sepia officinalis*. Uptake of ^{14}C-PCB#153 from seawater in the different body compartments and in whole-body juvenile cuttlefish (mean ng g^{-1} total lipids $\pm$ SD; $n = 3$).

the remaining tissues and in whole organisms (Fig. 6, Table 1). The separate body compartments (except cuttlebone) displayed PCB concentrations and transfer factors of the same order of magnitude (Fig. 6, Table 2).

As expected, the distribution of the contaminant among cuttlefish tissues determined at different times showed that at the beginning of the experiment, the ingested radiotracer was entirely associated with the digestive gland (Fig. 7). Over time, the proportion of ^{14}C-PCB activity significantly decreased in the digestive gland and increased in the remaining tissues (G test, $p < 0.05$).

5. Discussion

Cephalopods are well known for their capacity to accumulate radioactive, metallic or organic contaminants to relatively high levels (see e.g. Ueda et al., 1979; Miramand and Bentley, 1992; Yamada et al., 1997; Bustamante et al., 2000). However, only few studies have aimed at investigating the concentrations and distribution of PCBs in these molluscs, and most of

these studies have only considered whole organisms as a source of contaminant for their predators (Tanabe et al., 1984; Kawano et al., 1986; Weisbrod et al., 2000, 2001). Some studies have employed cephalopods as bioindicators of local or global PCB contamination (Butty and Holdway, 1997; Yamada et al., 1997), and others have demonstrated cellular or tissue effect of organic pollutants (Mann et al., 1988; Cheah et al., 1995). Nevertheless, a review of available literature clearly shows a general lack of information regarding bioaccumulation processes in cephalopods.

The present experimental work, exposing the common cuttlefish *S. officinalis* to environmentally realistic concentrations of a radiolabelled PCB congener, demonstrated the potential of these organisms to bioconcentrate PCBs to high levels. The bioconcentration efficiency was far greater when cuttlefish were exposed through seawater compared to either the food or sediment pathways. Indeed, PCB#153 concentration factors (CFs) from seawater were two to three orders of magnitude higher than transfer factors from sediment or food. These differences are in close agreement with observations from sea stars and sea urchins exposed to

Table 1
Sepia officinalis: parameters and statistics of the equations describing the uptake of ^{14}C-PCB #153 in different body compartments of the cuttlefish exposed via seawater, sediments and food

Body compartment	Model	C_0 (ASE)	C_{ss} (ASE)	k (ASE)	I (ASE)	R^2
Seawater						
Digestive gland	L	0		1.25 (0.05)		0.92
Cuttlebone	–					
Remaining tissues	L	0		7.45 (0.22)		0.95
Whole body	L	3.86 (3.76)		6.36 (0.38)		0.94
Sediments						
Digestive gland	C		6.94 (0.52)	4.40 (16.6)	2.22 (0.82)	0.75
Cuttlebone	–					
Remaining tissues	S		47.9 (3.11)	0.44 (0.09)		0.78
Whole body	S		39.0 (1.94)	0.49 (0.08)		0.77
Food						
Digestive gland	C		6.71 (0.44)	0.97 (0.55)	2.85 (0.54)	0.82
Cuttlebone	–					
Remaining tissues	L	−1.14 (0.69)		1.25 (0.07)		0.91
Whole body	L	−1.28 (0.64)		0.94 (0.07)		0.91

L (linear model), $C(t) = C_0 + kt$; S (saturation model), $C(t) = C_{ss}(1 - e^{-kt})$; C (combined model), $C(t) = C_{ss}(1 - e^{-kt})/(1 + e^{-k(t-I)})$; C_0, $C(t)$, C_{ss}, PCB concentrations (ng g^{-1} lipids) at, respectively, time 0, time t (d) and steady-state; k, rate constant (d^{-1}); I, time (d) at the inflexion point; ASE, asymptotic standard error; R^2, corrected determination coefficient.

the same contaminant under similar conditions (Danis et al., 2003, in press).

The distribution of the radiolabelled PCB congener was examined in three body compartments (the digestive gland, cuttlebone and the remaining tissues of juvenile cuttlefish) and the design of the experiments allowed following the uptake kinetics of the PCB in the separate body compartments. The digestive gland was selected, since this organ plays a major role in the energetic metabolism of cephalopods (Boucaud-Camou and Boucher-Rodoni, 1983) and has been documented to accumulate PCBs to high levels (Yamada et al., 1997; Ueno et al., 2003). The cuttlebone was also examined as this calcareous compartment acts as an internal skeleton and represents ca. 3% of the total body weight of the juveniles. Finally, the third compartment (remaining tissues) comprises the rest of the animal (ca. 90% of the total body weight) and mainly consists of muscles. The tracer experiments showed a similar distribution pattern between the three compartments irrespective of the

source of exposure: fractions of the radiolabelled PCB were always much higher in the remaining tissues, followed by the digestive gland and with very little amounts in the cuttlebone. Following exposure via seawater, sediments and food, the cuttlebone did not significantly concentrate the PCB congener. In fact, the cuttlebone contained less than 2.5% of the total contaminant load and only for a limited period of time. This is likely due to the internal localization of the cuttlebone which appears to have no direct contact with ambient seawater and to the low fat content of this tissue.

In contrast to the cuttlebone, the digestive gland readily accumulates PCB#153. The highest concentrations in the digestive gland were reached following seawater exposure. After 17 d of exposure, juvenile cuttlefish continued to concentrate the PCB in their digestive gland in a linear fashion. On the other hand,

Table 2
Sepia officinalis: concentration factors and transfer factors (CF, TF; mean ± SD; $n = 3$) in the body compartments and in whole-body juvenile cuttlefish at the end of the experimental exposures

	Digestive gland	Cuttlebone	Remaining tissues	Whole body
Seawater	527 ± 120	37.0 ± 24.7	3280 ± 513	2990 ± 449
Sediments	0.74 ± 0.26	0.00 ± 0.00	5.31 ± 0.77	4.65 ± 0.47
Food	1.81 ± 0.24	0.01 ± 0.03	3.72 ± 0.71	3.45 ± 0.61

CFs are calculated as the ratio between PCB#153 concentration in the body compartments (ng g^{-1} total lipids) and its concentration in seawater (ng g^{-1}). TFs are calculated as the ratio between PCB#153 concentration in the body compartments (ng g^{-1} total lipids) and its concentration in sediments (ng g^{-1} dry wt) or food (ng g^{-1} lipids).
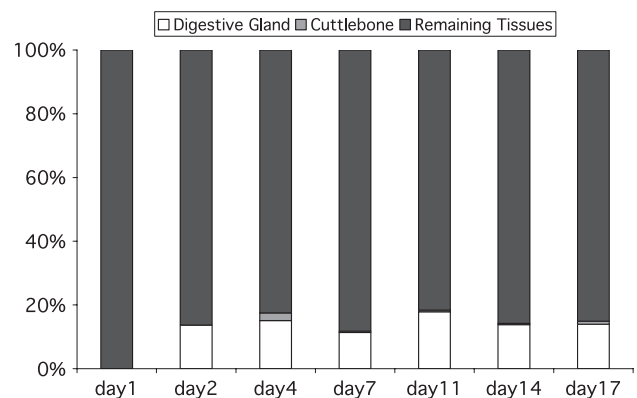

Fig. 3. *Sepia officinalis*. ^{14}C-PCB#153 distribution (mean %) among the different body compartments during the seawater experiment.
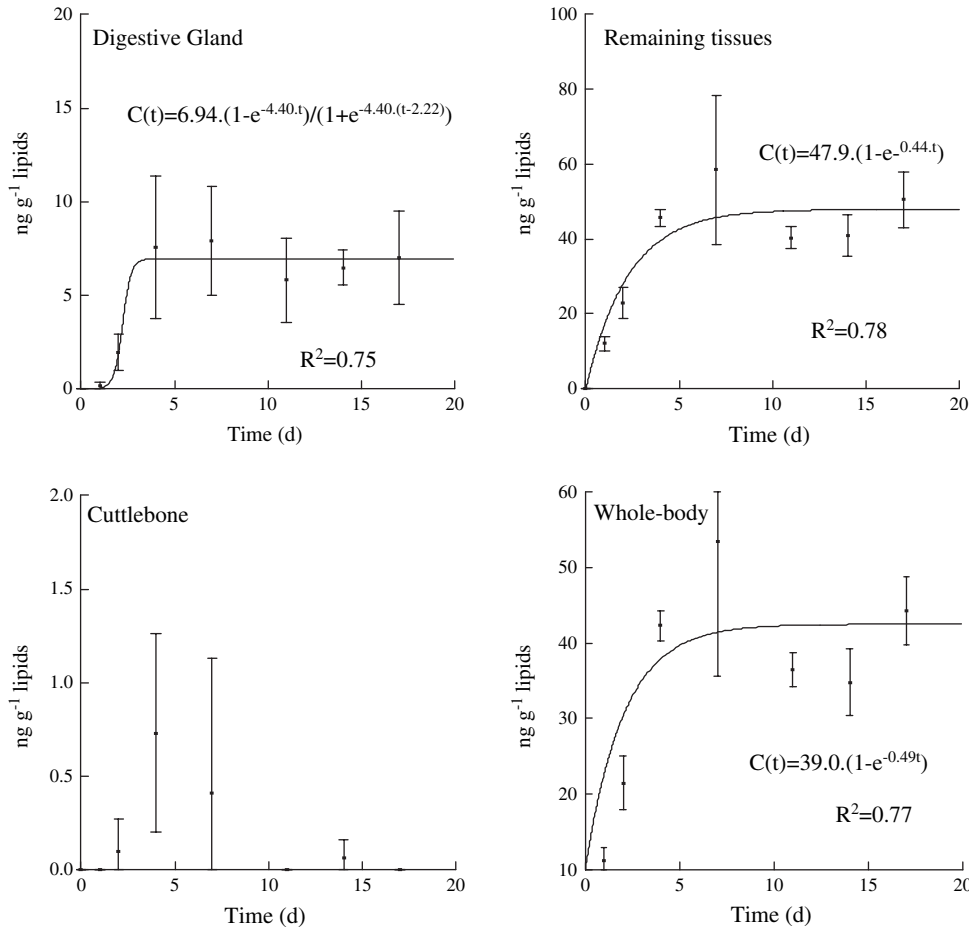
Fig. 4. *Sepia officinalis.* Uptake of ^{14}C-PCB#153 from sediments in the different body compartments and in whole-body juvenile cuttlefish (mean ng g^{−1} total lipids ± SD; $n = 3$).

PCB#153 uptake in the digestive gland displayed saturation kinetics following both sediment and food exposures. These results are particularly surprising for the digestive gland as it (a) has no direct contact with ambient seawater and (b) plays a major role in the digestive processes, including nutrient absorption. How-

Fig. 5. *Sepia officinalis.* ^{14}C-PCB#153 distribution (mean %) among the different body compartments during the sediment experiment.

ever, this organ is also involved in detoxification processes of xenobiotics in cephalopods (Cheah et al., 1995; Bustamante et al., 2002). Therefore, elevated concentrations in the digestive gland could result more from transfers from other organs accumulating compounds to be detoxified than from actual bioconcentration in the digestive gland itself. At this stage, it is difficult to explain the precise role of the cuttlefish digestive gland in the metabolism of PCBs; however, complex PCB redistribution processes clearly do occur among the tissues of this cephalopod. Therefore, it would be of major interest to characterize precisely the distribution of PCBs in adult cephalopods by making a finer separation of all tissues and organs.

Among the three compartments examined in these experiments, most of the PCB#153 taken up ($\geq 80\%$) was located in the remaining tissues regardless of the exposure pathway. However, the PCB concentration factors (CFs) calculated in the remaining tissues following the seawater experiment was three orders of magnitude higher than transfer factors (TFs) computed at the end of the sediment or food experiments. The fact that the remaining tissues are in direct contact with
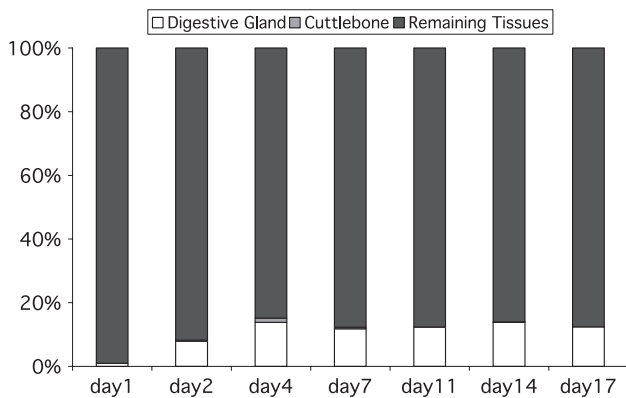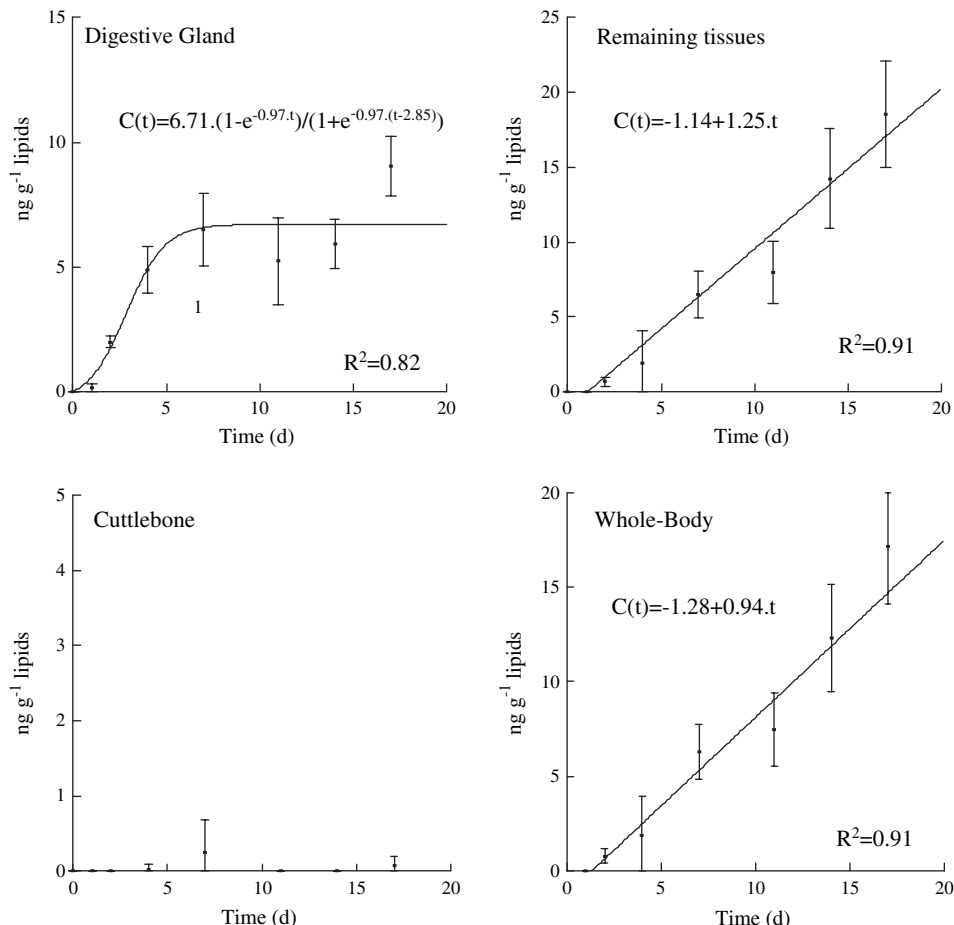
Fig. 6. *Sepia officinalis.* Uptake of ^{14}C-PCB#153 in the different body compartments and in whole-body juvenile cuttlefish (mean ng g^{-1} total lipids $\pm$ SD; $n = 3$) following ingestion of radiolabelled food (*Artemia salina*).

seawater could explain such a very high degree of accumulation following seawater exposure. However, cuttlefish also spend a substantial part of their time in very close contact with sediments; therefore, differences between CFs and TFs most probably also reflect differences in PCB bioavailability between the seawater and sediment pathways.

Fig. 7. *Sepia officinalis.* ^{14}C-PCB#153 distribution (mean %) among the different body compartments during the food experiment.

The uptake kinetics of PCB#153 in the remaining tissues followed a linear model when cuttlefish were exposed via seawater or food, and a saturation model when exposed via sediments. Saturation concentrations were relatively low if compared to studies with sea stars exposed to sediments spiked with similar PCB#153 concentrations (Danis et al., 2003). Sea stars displayed the same uptake kinetics but reached much higher PCB#153 concentrations, especially in compartments in direct contact with sediments ($\geq$3000 ng g^{-1} lipids in the body wall).

6. Conclusions

Although the cuttlefish used in this study were early juveniles, the results following seawater, sediment and food exposures have shown that PCBs are incorporated to high levels in their tissues. Even if this study was a first approach to understanding the pathways of PCB contamination in cephalopods, it highlights the general lack of knowledge concerning mechanisms underlying PCB bioaccumulation, distribution and depuration.
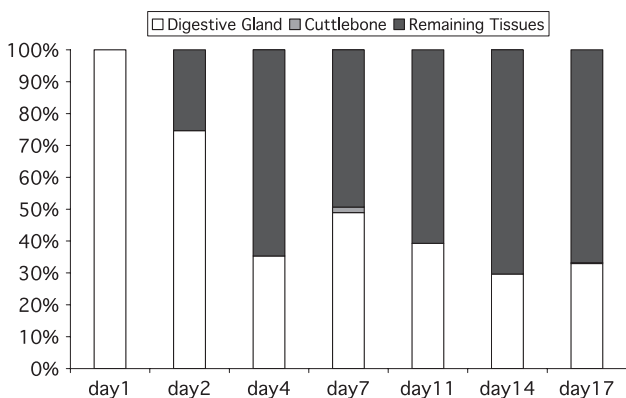
Finally, the main finding from this study is that seawater appears to be the main route for PCB incorporation in juvenile cuttlefish. Therefore, cuttlefish might be useful bioindicators of ambient water PCB contamination. Assuming that cuttlefish are representative of cephalopods in general, many species from contaminated areas could play a major role in the transfer of these persistent organic pollutants to their predators.

Acknowledgements

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