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Bioaccumulation and metabolisation of ^{14}C-pyrene by the Pacific oyster *Crassostrea gigas* exposed via seawater

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ABSTRACT

The first objective of this study was to determine the bioaccumulation kinetics of pyrene in the soft tissues of *Crassostrea gigas* (mantle, muscle, gills, digestive gland, and the remaining soft tissues). As bivalves can biotransform hydrocarbons in more polar compounds (metabolites) that are more easily excreted, the second objective was to investigate the oyster capacity to metabolize pyrene into its metabolite, the 1-hydroxypyrene. To these ends, oysters were exposed 24 h to waterborne ^{14}C-pyrene then placed in depuration conditions for 15 d. Oysters efficiently bioaccumulated pyrene in their soft tissues and equilibrium was reached within the exposure time. The metabolite1-hydroxypyrene was also detected in oyster tissues but represented only 4–14% of the parent pyrene. At the end of the exposure period, the gills and the mantle showed the highest pyrene proportion of total soft tissue content, i.e. 47% and 26%, respectively. After 15 d of depuration, the mantle contained 32% and 30% of the remaining pyrene and 1-hydroxypyrene, respectively. As *C. gigas* did not display a high capacity for metabolizing pyrene, it can be considered as a good bioindicator species to survey and monitor pyrene contamination in the coastal marine environment.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are found in many common products, such as petrol, fumes and household heating (e.g. using oil, gas or wood). Petroleum spills and discharges, seepages, industrial and municipal wastewater, urban and suburban surface run-off, and atmospheric deposition contribute to aquatic contamination caused by PAHs (Eisler, 1987). During the last decades, many studies have monitored the inputs, fluxes and fate of PAHs in the marine environment (Obana et al., 1983; Baumard et al., 1999). However, in order to assess the state of the marine ecosystem, it is necessary to know the fraction of these compounds which can be taken up by aquatic biota and their potential toxic effects (Escartin and Porte, 1999).

The quality of aquatic environments can be assess through the analysis of organisms considered as indicators of pollution, such as oysters, mussels and other bivalve molluscs (Pereira et al., 1992; Jaffé et al., 1995; Lauenstein, 1995; Beliaeff et al., 1997; Gunther et al., 1999). Indeed, because of their biological (e.g. capacity of bioaccumulation, resistance to physico-chemical stresses) and ecological characteristics (e.g. worldwide distribution, abundance of their populations), bivalves are among the best candidates to be bioindicator species (Phillips, 1976; Farrington and Tripp, 1993). These organisms can bioaccumulate a large variety of pollutants at levels higher than those present in the surrounding waters or sediments, and their behaviour can be recorded in short periods of time (Baumard et al., 1998; Solé et al., 2000). In this way, mussels have been extensively used worldwide as sentinel organisms to monitor the uptake and accumulation of PAHs in the coastal environments, in the Mussel Watch Program in the USA (O'Connor, 1996; O'Connor and Lauenstein, 2006), the Coordinated Environmental Monitoring Program (OSPAR, 2010) and the Réseau National de la Contamination CHimique (ROCCH, 2008) in France. In this last country, oysters are also used as a sentinel species in the ROCCH monitoring program, which is particularly relevant in areas where mussels are absent.

The exposure of marine organisms to PAHs has often been evaluated by measuring tissue contaminant contents (Varanasi et al., 1989). However, when considering biotransformation capacities of these organisms, this approach becomes less relevant. Indeed, biotransformation refers to the entire modification of chemical molecules occurring in the organisms. Metabolism of PAHs in marine invertebrates is apparently related to cytochrome P450 (EC 1.14.14.1). This enzyme converts parent hydrophobic and lipid-soluble PAHs, into water soluble metabolites. However, the mechanisms by which the involved enzymes are regulated are still poorly understood (Hahn, 1998). In marine mammals and birds,

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some studies have shown that the low concentrations of PAHs in their tissues were due to a combination of inefficient bioaccumulation from food and rapid metabolisation and excretion of accumulated PAHs (Watanabe et al., 1989; Fossi et al., 1995). In the common sole *Solea solea* affected by a strong PAH pollution, the liver produced metabolites that were released through the bile (Budzinski et al., 2004). Previously, it was believed that molluscs possessed a weak to non-existent ability to metabolize PAHs (Lee et al., 1972a; Palmork and Solbakken, 1981). Conversely, molluscs may have relatively strong metabolisation systems (McElroy et al., 2000) and it has been shown that molluscs collected in strongly polluted environments contained often low concentrations of PAHs (e.g. Varanasi et al., 1989; Baumard, 1997).

The Pacific oyster *Crassostrea gigas* (Thunberg, 1793) has a high economic value in the world and especially in France. Indeed, most of French oyster-farming raise this species which production averages up to 128 000 tonnes per year (CNC, 2004). Many farmhouses exist all along the French coastline and they are very sensitive to marine contamination, especially during oil slicks. It is therefore fundamental to better understand the mechanisms of bioaccumulation of PAHs in this species.

Various studies have shown that pyrene and other PAHs with four benzene rings are among the most predominant PAHs in bivalves (Obana et al., 1983; Varanasi et al., 1985; Wade et al., 1988; Kaag et al., 1997). Moreover, pyrene is considered as one of the 16 most toxic contaminants for the environment and the dominant PAHs in the marine environment (Giessing et al., 2003). Therefore, the first aim of this study was to follow the kinetics of uptake and depuration of pyrene in the organs and tissues of *C. gigas* exposed via seawater. In order to study environmentally realistic contaminant levels, the pyrene used was ^{14}C-labelled and measured using highly sensitive radiodetection technique. The second objective of this work was to determine the presence and the kinetics of formation of 1-hydroxypyrene, i.e. the pyrene metabolite previously demonstrated to be predominant in fish bile (e.g. Krahn et al., 1987; Ariese et al., 1993).

2. Materials and methods

2.1. Biological material

Oysters were purchased from a shellfish farm on the French Atlantic coast (La Rochelle). Organisms were then transferred to the Environment Laboratories premises (IAEA, Monaco). Prior to the experimentation, specimens were acclimated to laboratory conditions for 2 months (constantly aerated open-circuit aquarium; salinity: 36 ± 1 p.s.u.; temperature: 19 ± 1 °C; pH: 8; light/dark cycle: 12 h/12 h). During acclimation, bivalves were fed phytoplankton using the Prymnesiophyceae *Isochrysis galbana* (10^4 cells mL^{-1}). Recorded mortality was lower than 5% over the acclimation period.

2.2. Radiotracer and radioanalyses

The ^{14}C-labelled 4, 5, 9, 10 pyrene was purchased from Sigma, USA. Specific activity was 2.17×10^9 Bq mmol^{-1}. Stock solutions were prepared in methanol and a final concentration of 27 μg L^{-1} was used.

Two milliliter of the mixtures containing pyrene and 1-hydroxypyrene or purified pyrene samples (see below) were transferred to 20 mL glass scintillation vials (Packard) and mixed with 10 mL of scintillation liquid (Ultima Gold, Packard). ^{14}C-radioactivity was measured using a 1600 TR Liquid Scintillation Analyser (Packard). Activity was determined by comparison with standards of known activities and measurements were corrected for counting efficiency and quenching effect. Counting time was adjusted to obtain a propagated counting error less than 5%.

2.3. Experimental procedure

2.3.1. Uptake phase

Forty four oysters were placed in a 50 L glass aquarium containing natural seawater (closed circuit) spiked with ^{14}C-labelled pyrene. The initial pyrene concentration in the aquarium was 0.27 μg L^{-1}. This concentration matches with PAHs values found in strongly polluted areas (Axelman et al., 1999). Every 30 min, seawater radioactivity was measured and pyrene was added as required in order to keep its concentration constant during the whole accumulation phase (24 h). Oysters were not fed during the exposure period. Four animals were collected at different times (0, 2, 5, 9, 19 and 24 h) in order to follow the uptake kinetics of ^{14}C-pyrene and the formation of its metabolite. At the end of the exposure period (24 h), seawater was sampled to detect whether metabolites could have been released by exposed animals.

At each sampling time, oyster soft tissues were dissected into five compartments: mantle, gills, muscle, digestive gland and remaining tissues (i.e., labial palps, gonad and heart). Each organ and tissue was weighed and crushed. Then, samples were treated with 200 μL of β-glucuronidase-aryl-sulfatase mixture containing 100 000 units mL^{-1} of glucuronidase (Sigma) and 7500 units mL^{-1} of sulfatase (Sigma) for enzymatic deconjugation. To this purpose, the samples were buffered to pH 5 with 3 mL of 5 M potassium acetate buffer. They were then placed in an oven at 37 °C during 20 h.

Pyrene and 1-hydroxypyrene were extracted via two subsequent liquid/liquid extractions. The first extraction was performed by adding 5 mL of a hexane/dichloromethane solution (50:50) and the second extraction was performed by adding 4 mL of a methanol/dichloromethane solution (10:90) to extract pyrene and 1-hydroxypyrene, respectively. For both extractions, the samples were mechanically shaken for 30 min and then centrifuged at 6000 rpm for 5 min. In the first extraction, the organic phase (5 mL) was recovered in a flat bottom flask. Following the second extraction, the organic phase was recovered and combined with the first one. Two milliliter of the extracted solution was radioanalysed to determine the amount of pyrene + 1-hydroxypyrene in each organ.

The rest of the mixture was concentrated under a gentle stream of nitrogen to 1 mL and then separated by using upti-clean SPE glass columns Si/Cn–S (Interchim, Montluçon, France). Pyrene was eluted with 5 mL of a hexane/dichloromethane (50:50) solution which was radioanalysed.

The 1-hydroxypyrene content in each organ was calculated by comparing the results of the two radioanalyses.

2.3.2. Depuration phase

At the end of the exposure period, the remaining organisms were placed in an open circuit 50 L seawater aquarium (salinity: 36 ± 1 p.s.u.; temperature: 19 ± 1 °C; pH: 8; light/dark cycle: 12 h/12 h). At different times of the depuration period (0, 6, 18, 36, 96,192 and 336 h) four oysters were collected and their soft tissues dissected in order to follow the variation in pyrene and its metabolite. The dissected tissues and organs were processed according to the same method as previously described.

2.4. Data analyses

2.4.1. Uptake kinetics

A first order model was used to assess changes in pyrene concentration in oyster tissues along time during the exposure to

waterborne pyrene. In this model the change in tissue activity with time was calculated by:

$$dA_{org}/dt = k_u A_{SW} - k_e A_{org} \quad (1)$$

where A_{org} is the activity of pyrene in tissue (Bq g^{-1} tissue), A_{SW} the activity of pyrene in seawater (Bq g^{-1} water), k_u the uptake rate constant (Bq g^{-1} h^{-1}), k_e the elimination rate constant (Bq g^{-1} h^{-1}) and t is the time (h).

As A_{SW} was maintained constant during the uptake experiment phase, Eq. (1) can be integrated to estimate tissue activities at any exposure time by:

$$A_{org}t = A_{SW}(k_u/k_e)(1 - e^{-k_e t}) \quad (2)$$

where $A_{org}t$ is the tissue activity at time t.

When steady-state tissue activities are attained (i.e. $dA_{org}/dt = 0$), the bioconcentration factor (BCF) can be estimated as follows:

$$A_{org}/A_{SW} = BCF = k_u/k_e \quad (3)$$

In this study, the BCF is the ratio between ^{14}C-labelled pyrene in the body and in the surrounding seawater.

2.4.2. Depuration kinetics

The depuration kinetics were best fitted using either a single-component exponential equation (Eq. (4)) or a double-component exponential equation (Eq. (5)):

$$A_t = A_0(e^{-k_e t}) \quad (4)$$

$$A_t = A_{0s}(e^{-k_{es} t}) + A_{0l}(^{e-k_{el} t}) \quad (5)$$

where A_t is the remaining activity at time t (Bq g^{-1}), A_0 the activity at time 0 (Bq g^{-1}), i.e. at the beginning of the depuration period and k_e is the depuration rate constant (Bq g^{-1} h^{-1}).

For double-component exponential model (Eq. (5)), a 'short-lived' component (s) and a long-lived' component (l) describe the radiotracer proportion that is depurated rapidly (s) and slowly (l), respectively. For each exponential component (s and l), a biological half-life can be calculated ($T_{b1/2s}$ and $T_{b1/2l}$) from the corresponding depuration rate constants (k_{es} and k_{el}, respectively) according to the relation:

$$T_{b1/2} = \ln 2/k_e \quad (6)$$

2.4.3. Statistical analysis

Model constants and their statistics were estimated by iterative adjustment of the model using the nonlinear curve-fitting routines in the Statistica 6 software. For depuration kinetics, best fitting models were selected between single- and double-component exponential equation, according to the highest determination coefficient and examination of residuals. The level of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Seawater exposure

Uptake of pyrene in five body compartments (mantle, gills, muscle, digestive gland and remaining tissues) of *C. gigas* exposed to spiked seawater for 24 h is presented in Fig. 1. The parameters and statistics of the uptake kinetic of ^{14}C-pyrene are summarised in Table 1. The steady-state BCF of pyrene in the whole soft parts of the oysters was observed within 24 h and reached 1560 ± 630 (Table 1). Among the tissues, the gills accumulated rapidly and strongly the contaminant with a BCF of 2080 ± 860. However, each organ accumulated the pyrene in a different way (Fig. 1). For example, during the uptake phase, the mantle and the remaining tissues efficiently accumulated waterborne pyrene but the state of equilibrium was not reached during the time frame of the experiment (Fig. 1).

The final distribution of the contaminant in each compartment after 24 h of exposure is presented in Fig. 2. Among tissues, gills contained half of the whole body burden radioactivity and consistently showed the highest rate of accumulation with a k_u of 433 Bq g^{-1} h^{-1} (Table 1). In contrast, the muscle was the organ with the lower rate of accumulation (k_u = 38 Bq g^{-1} h^{-1}) and thus, only contained a very low proportion (5%) of the total quantity of ^{14}C-pyrene present in oyster soft tissues (Fig. 2).

Quantification of the pyrene metabolites was carried out at each sampling time, and the proportion of 1-hydroxypyrene represented between 4% and 14% of the total radioactivity (Fig. 3).

At the end of the exposure period, the concentration of 1-hydroxypyrene in seawater was below the detection limit of the method.

3.2. Depuration phase

At the end of the exposure time, non-contaminating conditions were restored and depuration kinetics of the pyrene were followed in the organs and tissues of the oysters for 15 d. The loss of incorporated ^{14}C-labelled pyrene followed a single or a double exponential model in the different body compartments (Fig. 4 and Table 2). In the muscle, gills and mantle, depuration was best described by a double-component exponential equation (R^2 = 0.42, 0.52 and 0.48, respectively). The resulting biological half-lives ($T_{b1/2}$) ranged from 1.17 (mantle) to 9.35 h (gills) for the short-lived compartment and from 2.75 (muscle) to 7.27 d (gills) for the long-lived compartment (Table 2). In contrast, a single-component exponential equation better fitted the depuration kinetics in the digestive gland and in the remaining tissues (R^2 = 0.37 and 0.30, respectively). They were characterised by a relatively strong retention of pyrene: the resulting $T_{b1/2}$ were 87.4 and 97.4 h, respectively, (i.e., 3.64 and 4.05 d) (Table 2 and Fig. 4).

The distribution of ^{14}C-labelled pyrene among the oyster soft tissues was determined at the end of the depuration period (Fig. 5). It differed from the distribution observed at the end of the exposure period (Fig. 2), with a lower fraction associated to the gills (24 ± 10 vs. 47 ± 13%) and a higher fraction associated to the digestive gland (19 ± 10 vs. 6 ± 4%) and the remaining tissues (21 ± 18 vs. 16 ± 3%). At the end of the depuration period, the digestive gland displayed the highest pyrene activity (data not shown). In contrast, the gills had lost 50% of their activity during the depuration phase. Fig. 5 also shows the distribution of 1-hydroxypyrene between the body compartments at the end of the depuration period. 1-hydroxypyrene was distributed in similar proportion as pyrene in the oyster body compartments.

4. Discussion

Oysters accumulated very efficiently the ^{14}C-labelled waterborne pyrene following a saturation model and after a short exposure period (24 h), organisms reached the state of equilibrium. Among soft tissues, the gills accumulated rapidly and strongly the contaminant with a BCF of 2080 (Table 1) likely because of the high filtration rate of oysters which could be as high as 3.9 L h^{-1} g^{-1} dry weight (Bougrier et al., 1995). Consequently, the gills displayed the highest activities at the end of the exposure phase. Absorption of pyrene onto gills might be facilitated in oysters, as it occurs in blue mussels *Mytilus edulis* which have a micellar layer which absorbs hydrocarbons (Lee et al., 1972a). Nevertheless, the accumulated pyrene was relatively rapidly lost

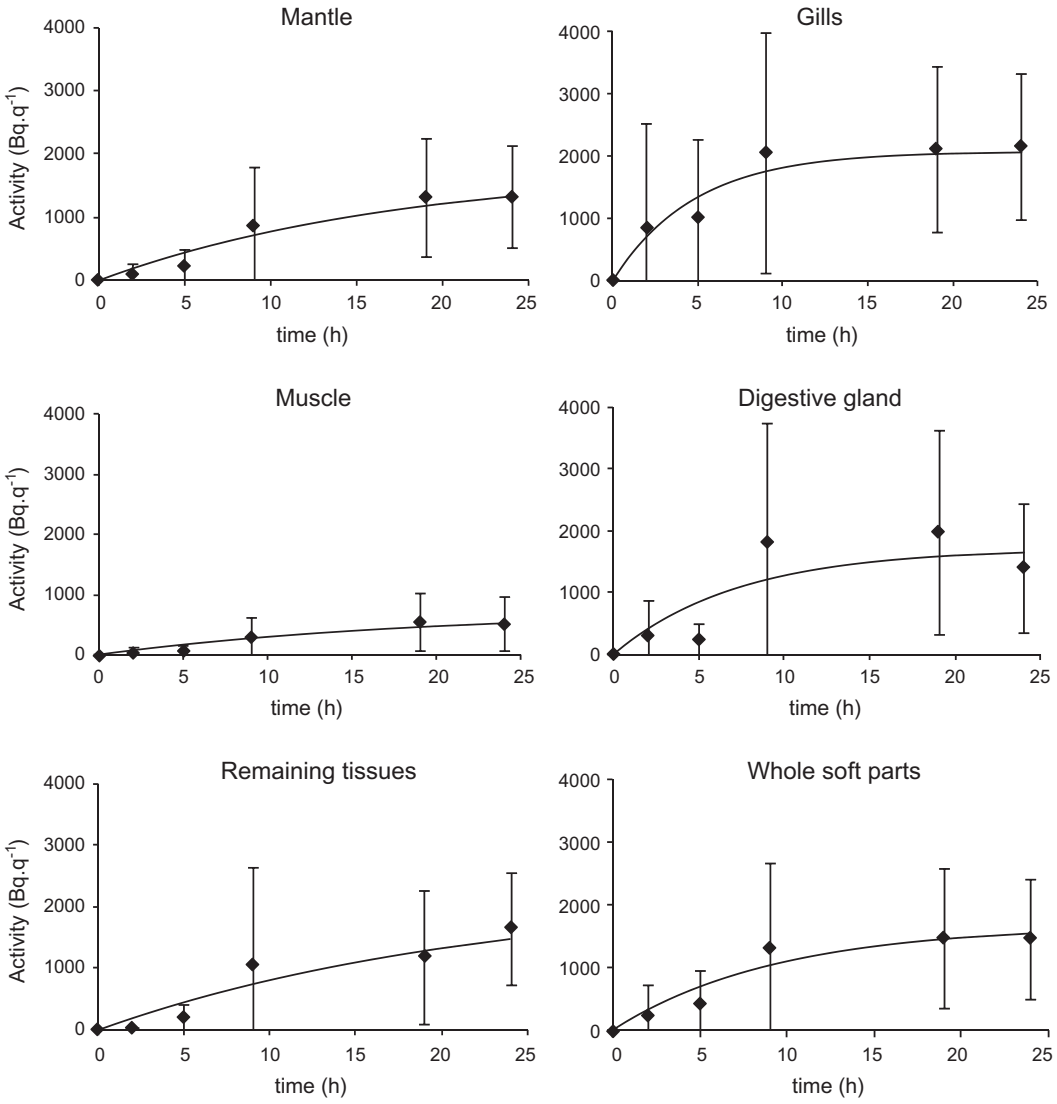

Fig. 1. Uptake kinetics of ^{14}C-labelled pyrene in five body compartments and in the whole soft parts of the oysters ($n = 4$) during the 24 h seawater contamination period. Parameters and statistics of the uptake kinetics are given in Table 1.

Table 1
Parameters of the equation describing the uptake of pyrene in the body compartments and in the whole soft parts of the oysters ($n = 4$) after 24 h seawater contamination (k_u = uptake rate constant; k_e = elimination rate constant; BCF = Bioconcentration factor).

Body compartment	k_u (Bq g^{-1} h^{-1})	k_e (Bq g^{-1} h^{-1})	BCF (k_u/k_e)	R^2
Mantle	102 ± 10	0.056 ± 0.006	1820 ± 530	0.45
Gills	433 ± 62	0.208 ± 0.030	2080 ± 860	0.28
Muscle	38 ± 6	0.051 ± 0.008	750 ± 380	0.38
Digestive gland	234 ± 50	0.137 ± 0.030	1710 ± 970	0.26
Remaining tissues	100 ± 12	0.045 ± 0.006	2220 ± 810	0.35
Whole body	158 ± 20	0.101 ± 0.014	1560 ± 630	0.39

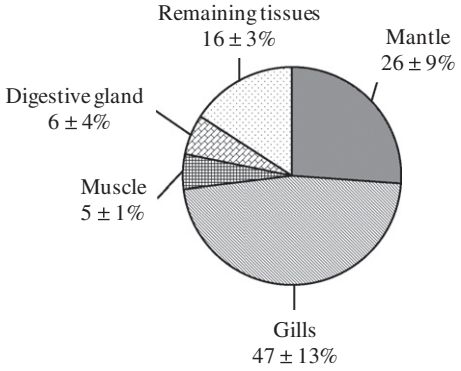

Fig. 2. Distribution of ^{14}C-labelled pyrene (%) among the body compartments of oysters ($n = 4$) at the end of the exposure period (24 h).

from this tissue (Table 1). Indeed, pyrene proportion in the gills at the end of the depuration phase was half than at the end of the exposure period (Figs. 2 and 5). This decrease is due to a fast depuration rate of pyrene in the gills (Table 1). At the same time, the increase of pyrene proportions in the digestive gland and remaining tissues suggest that it was transferred towards from the gills to these tissues as previously reported in other organisms (Neff, 1979). Therefore, the decrease of pyrene observed in the gills was also due to a relative increase of the activity in other compartments, such as the digestive gland (data not shown).

During the uptake phase, the mantle and the remaining tissues also accumulated pyrene but the steady-state was not reached for these compartments. This is probably due to the fact that, even if

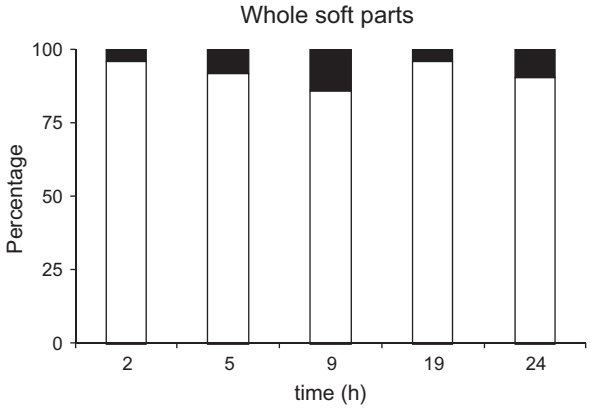

Fig. 3. Proportion (%) between ^{14}C-labelled pyrene (in white) and 1-hydroxypyrene (in black) in the whole soft parts of the oysters ($n = 4$) at the end of the exposure period (24 h).

these tissues are in contact with seawater, their surface is much smaller than that of the gills, and the cell types and the thickness of the epithelium differ completely (Auffret, 2003). The resulting uptake rates (k_u) were lower for both tissues than for gills. During the depuration phase, the mantle released pyrene quickly. In contrast, the remaining tissues showed a slow increase of the pyrene proportion against the total pyrene content in oysters as well as of its metabolite proportion (against the total metabolite content in oysters) during the depuration period. It is well-known that tissues rich in lipids, e.g. gonads, accumulate preferentially PAHs because of the highly hydrophobic nature of the latter (Meador et al., 1995; Berthelin et al., 2000). Moreover, a strong increase of the activity in the remaining tissues was noticed during the third sampling in the depuration period ($t = 36$ h). The four oysters studied at this moment showed the particularity of being in the reproduction stage and of having gonadic tissues more abundant than the average of other individuals. For this reason, having acknowledged the unlikely hypothesis that these observations could come partly from an error of manipulation, it seems that this increase of activity was rather due to a stronger retention in mature gonadic tissues. This conclusion is consistent with results of Ellis et al. (1993), where gonads from *Crassostrea virginica* displayed PAH concentrations five times higher than in somatic tissues.

In contrast, the digestive gland and the muscle which are not truly in direct contact with seawater displayed lower BCF than the gills, the mantle and the remaining tissues (Table 1). Neverthe-

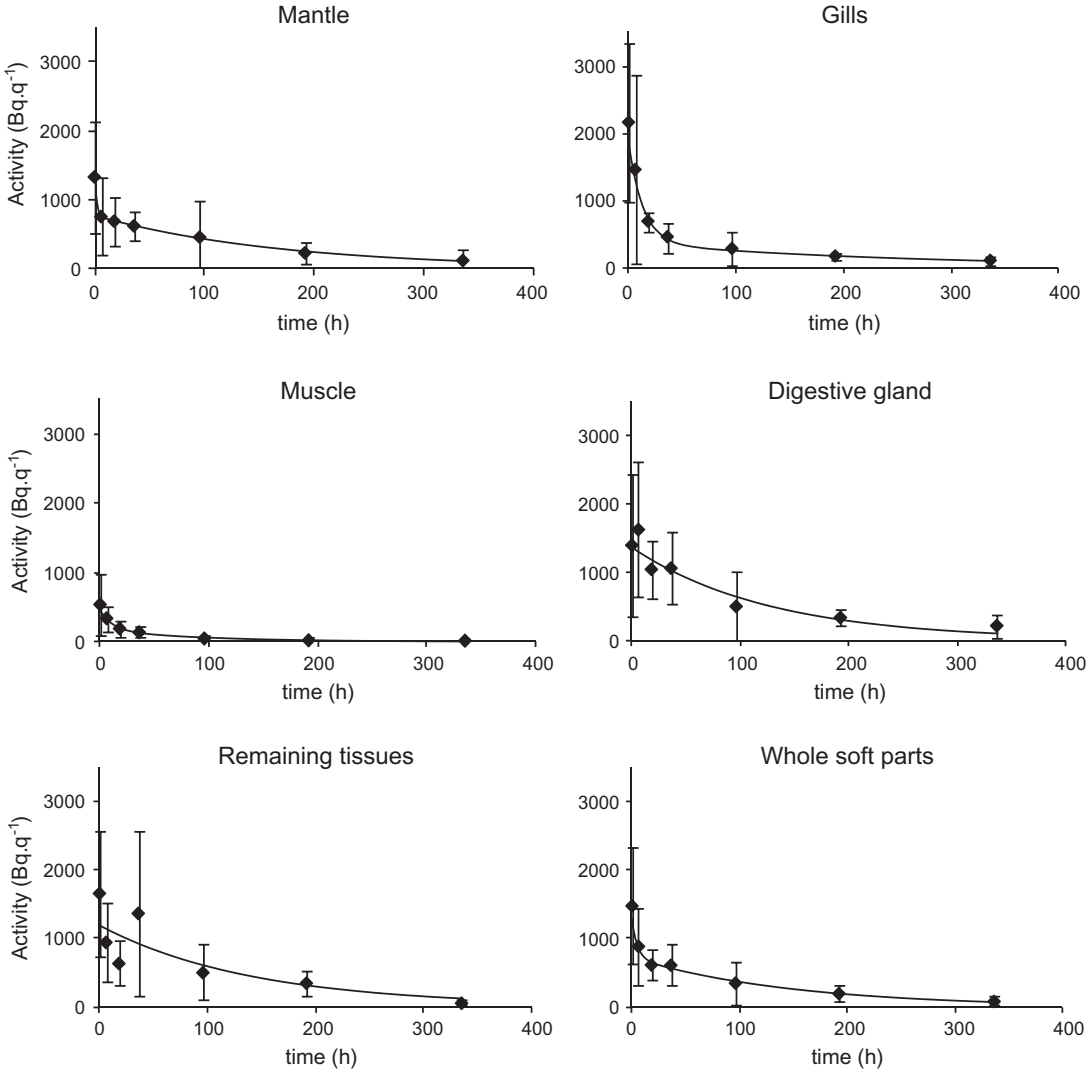

Fig. 4. Loss kinetics of ^{14}C-labelled pyrene in five body compartments and in the whole soft parts of the oysters ($n = 4$) after a 24 h seawater exposure. Parameters and statistics of the loss kinetics are given in Table 2.

Table 2
Parameters of the equations describing the loss kinetics of pyrene in the different oyster body compartments in the whole soft tissues ($n = 4$) after a 24 h exposure from seawater. O and T: 1- and 2-exponential loss equations, respectively; A_0: remaining activity at time 0; k_e: depuration rate constant; s: short and l; long-lived elimination, respectively; R^2: determination coefficient; $T_{b½}$: biological half-life in hours (h) or days (d). For abbreviation definitions, see 'Data and statistical analyses'.

Compartment	Model	A_{0s}	k_{es}	$T_{b1/2s}$ (h)	A_{0l}	k_{el}	$T_{b1/2l}$ (d)	R^2	p
Mantle	T	443	0.591	1.17	769	0.006	4.92	0.42	<0.001
Gills	T	1578	0.074	9.35	368	0.004	7.27	0.52	<0.001
Muscle	T	291	0.087	7.93	166	0.011	2.75	0.48	<0.001
Digestive gland	O	1360	0.008	87.4				0.37	<0.001
Remaining tissues	O	1203	0.007	97.4				0.30	<0.001
Whole soft parts	T	600	0.195	3.55	721	0.007	4.29	0.53	<0.001

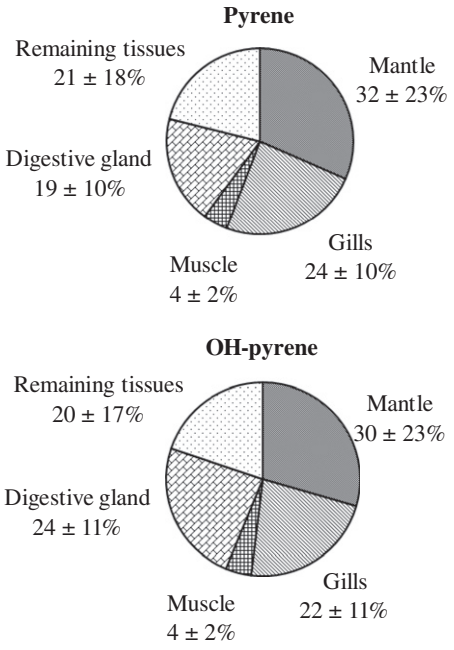

Fig. 5. Distribution (%) of ^{14}C-labelled pyrene and 1-hydroxypyrene among the different body compartments of the oysters ($n = 4$) at the end of the depuration period (15 d).

less, the uptake rate for the digestive gland was much higher than the one of the muscle. The steady-state of pyrene in the digestive gland was reached very quickly, i.e. after 9 h while it took 19 h in the muscle (Fig. 1). The results strongly suggest that during the exposure period pyrene was transferred from tissues in contact with seawater such as gills, towards the digestive gland. Moreover, the digestive gland showed a higher percentage of 1-hydroxypyrene (19%) in relation to total radioactivity (pyrene + 1-hydroxypyrene) than the other organs (9–12%) and the proportion of pyrene and its metabolite in the digestive gland increased from 6% to 10% at the end of the accumulation period to 19–24% at the end of depuration period (Figs. 2 and 5). This suggests that the digestive gland had a stronger metabolisation capacity of pyrene than the other tissues.

Among all the tissues studied, the digestive gland and the remaining tissues displayed the strongest retention capacity with $T_{b½}$ of 87.4 and 97.4 h, respectively. Indeed, just like almost all organic contaminants, PAHs best store up in some tissues, namely in the hepatopancreas in invertebrates and in the liver in vertebrates (Meador et al., 1995). Because of their highly hydrophobic nature, PAHs are mostly accumulated in tissues with high lipid contents. The transformed PAH metabolites generally accumulate in the hepatopancreas (Lee et al., 1976; Neff et al., 1976; Meador et al., 1995). It has been proved that for some invertebrates and for several fish species exposed to PAHs either via water, food or sediments, the cytochrome P450 enzymatic system connected to an oxygenase function (MFO) is activated in their hepatic structure (Andersson and Forlin, 1992). In the case of fish, this enzymatic system allows excreting most of the PAHs bioaccumulated via the bile and the urine (Pritchard and Bend, 1991). This particular function was difficult to bring to light concerning bivalves. For a long time, it was admitted that bivalves did not possess a P450 system (Lee et al., 1972b; Vandermeulen and Penrose, 1978). However, more recent studies have shown that bivalves do possess a P450 system (Lake et al., 1985; McLeese and Burridge, 1987), allowing them to metabolize PAHs. As it is the case for the majority of marine invertebrates, such ability to metabolize PAHs is weaker than for vertebrates (Livingstone, 1994; Stegeman and Hahn, 1994).

Data obtained on 1-hydroxypyrene in the tissues and organs of *C. gigas* seem to confirm that metabolisation of pyrene in oysters is relatively weak and therefore would not be the driving mechanism responsible for its elimination. It is however possible that because of the large volumes of water they filter continuously, water-soluble metabolites would be rapidly excreted and, therefore, not accumulated in the tissues (James, 1989). If the event lasts only for few days, it is very likely that most of the accumulated PAHs are going to be eliminated. In contrast, Meador et al. (1995) suggested that, during a chronic exposure, a non negligible fraction of the PAHs could be stored in lipids and become less subject to the elimination by diffusion or by metabolisation.

In conclusion, *C. gigas* presents a good potential of bioaccumulation of the pyrene and seems to have a low metabolisation capacity for this compound. Such a bioaccumulation capacity suggests that *C. gigas* could be used as a valuable bioindicator for pyrene. Following a marine contamination, oysters are thus able to rapidly accumulate the contaminant in detectable concentrations. Besides, as this species is very common in several regions of the world, samples can be collected at any time and in many country. This makes the situation easier for comparing data. However, its speed of depuration is also quick, thus it does not allow the recording of long-term pollution. On the contrary, it is very interesting for the monitoring of the pollution variations on short-term periods.

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