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

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1 Bioaccumulation and metabolisation of <sup>14</sup>C-pyrene by the Pacific oyster  
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3

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

34

35 **Keywords:** polycyclic aromatic hydrocarbons; bivalve; tissue distribution; bioaccumulation;  
36 kinetics

37

## 38 1. Introduction

39 Polycyclic aromatic hydrocarbons (PAHs) are found in many common products, such as  
40 petrol, fumes and household heating (e.g. using oil, gas or wood). Petroleum spills and  
41 discharges, seepages, industrial and municipal wastewater, urban and suburban surface run-  
42 off, and atmospheric deposition contribute to aquatic contamination caused by PAHs (Eisler,  
43 1987). During the last decades, many studies have monitored the inputs, fluxes and fate of  
44 PAHs in the marine environment (Obana et al. 1983; Baumard et al. 1999). However, in order  
45 to assess the state of the marine ecosystem, it is necessary to know the fraction of these  
46 compounds which can be taken up by aquatic biota and their potential toxic effects (Escartin  
47 and Porte, 1999).

48 The quality of aquatic environments can be assess through the analysis of organisms  
49 considered as indicators of pollution, such as oysters, mussels and other bivalve molluscs  
50 (Pereira et al. 1992; Jaffé et al. 1995; Lauenstein, 1995; Beliaeff et al. 1997; Gunther et al.  
51 1999). Indeed, because of their biological (e.g. capacity of bioaccumulation, resistance to  
52 physico-chemical stresses) and ecological characteristics (e.g. worldwide distribution,  
53 abundance of their populations), bivalves are among the best candidates to be bioindicator  
54 species (Phillips, 1976; Farrington and Tripp, 1993). These organisms can bioaccumulate a  
55 large variety of pollutants at levels higher than those present in the surrounding waters or  
56 sediments, and their behaviour can be recorded in short periods of time (Baumard et al. 1998;  
57 Solé et al. 2000). In this way, mussels have been extensively used worldwide as sentinel  
58 organisms to monitor the uptake and accumulation of PAHs in the coastal environments, in  
59 the Mussel Watch Program in the USA (O'Connor 1996, O'Connor and Lauenstein 2006), the  
60 Coordinated Environmental Monitoring Program (OSPAR 2010) and the Réseau National de  
61 la Contamination CHimique (ROCCH 2008) in France. In this last country, oysters are also

62 used as a sentinel species in the ROCCH monitoring program, which is particularly relevant  
63 in areas where mussels are absent.

64 The exposure of marine organisms to PAHs has often been evaluated by measuring tissue  
65 contaminant contents (Varanasi et al. 1989). However, when considering biotransformation  
66 capacities of these organisms, this approach becomes less relevant. Indeed, biotransformation  
67 refers to the entire modification of chemical molecules occurring in the organisms.  
68 Metabolism of PAHs in marine invertebrates is apparently related to cytochrome P450 (EC  
69 1.14.14.1). This enzyme converts parent hydrophobic and lipid-soluble PAHs, into water  
70 soluble metabolites. However, the mechanisms by which the involved enzymes are regulated  
71 are still poorly understood (Hahn, 1998). In marine mammals and birds, some studies have  
72 shown that the low concentrations of PAHs in their tissues were due to a combination of  
73 inefficient bioaccumulation from food and rapid metabolisation and excretion of accumulated  
74 PAHs (Watanabe et al. 1989; Fossi et al. 1995). In the common sole *Solea solea* affected by a  
75 strong PAH pollution, the liver produced metabolites that were released through the bile  
76 (Budzinski et al. 2004). Previously, it was believed that molluscs possessed a weak to non-  
77 existent ability to metabolize PAHs (Lee et al. 1972a; Palmork and Solbakken, 1981).  
78 Conversely, molluscs may have relatively strong metabolisation systems (McElroy et al.  
79 2000) and it has been shown that molluscs collected in strongly polluted environments  
80 contained often low concentrations of PAHs (e.g. Varanasi et al. 1989; Baumard, 1997).

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

87 Various studies have shown that pyrene and other PAHs with four benzene rings are among  
88 the most predominant PAHs in bivalves (Obana et al. 1983; Varanasi et al. 1985; Wade et al.  
89 1988; Kaag et al. 1997). Moreover, pyrene is considered as one of the 16 most toxic  
90 contaminants for the environment and the dominant PAHs in the marine environment  
91 (Giessing et al. 2003). Therefore, the first aim of this study was to follow the kinetics of  
92 uptake and depuration of pyrene in the organs and tissues of *C. gigas* exposed via seawater. In  
93 order to study environmentally realistic contaminant levels, the pyrene used was <sup>14</sup>C-labelled  
94 and measured using highly sensitive radiodetection technique. The second objective of this  
95 work was to determine the presence and the kinetics of formation of 1-hydroxypyrene, i.e. the  
96 pyrene metabolite previously demonstrated to be predominant in fish bile (e.g. Krahn et al.  
97 1987; Ariese et al. 1993),

98

## 99 **2. Materials and methods**

### 100 **2.1. Biological material**

101 Oysters were purchased from a shellfish farm on the French Atlantic coast (La Rochelle).  
102 Organisms were then transferred to the Environment Laboratories premises (IAEA, Monaco).  
103 Prior to the experimentation, specimens were acclimated to laboratory conditions for two  
104 months (constantly aerated open-circuit aquarium; salinity:  $36 \pm 1$  p.s.u.; temperature:  $19 \pm 1$   
105 °C; pH: 8; light/dark cycle: 12h/12h). During acclimation, bivalves were fed phytoplankton  
106 using the Prymnesiophyceae *Isochrysis galbana* ( $10^4$  cells ml<sup>-1</sup>). Recorded mortality was  
107 lower than 5% over the acclimation period.

108

### 109 **2.2. Radiotracer and radioanalyses**

110 The <sup>14</sup>C-labelled 4, 5, 9, 10 pyrene was purchased from Sigma, USA. Specific activity was  
111  $2.17 \cdot 10^9$  Bq mmol<sup>-1</sup>. Stock solutions were prepared in methanol and a final concentration of  
112  $27 \mu\text{g L}^{-1}$  was used.

113 Two mL of the mixtures containing pyrene and 1-hydroxypyrene or purified pyrene samples  
114 (see below) were transferred to 20 mL glass scintillation vials (Packard) and mixed with 10  
115 mL of scintillation liquid (Ultima Gold, Packard). <sup>14</sup>C-radioactivity was measured using a  
116 1600 TR Liquid Scintillation Analyser (Packard). Activity was determined by comparison  
117 with standards of known activities and measurements were corrected for counting efficiency  
118 and quenching effect. Counting time was adjusted to obtain a propagated counting error less  
119 than 5%.

120

## 121 **2.3. Experimental procedure**

### 122 2.3.1. Uptake phase

123 Forty four oysters were placed in a 50L glass aquarium containing natural seawater (closed  
124 circuit) spiked with <sup>14</sup>C-labelled pyrene. The initial pyrene concentration in the aquarium was  
125 0.27 µg L<sup>-1</sup>. This concentration matches with PAHs values found in strongly polluted areas  
126 (Axelman et al. 1999). Every 30 minutes, seawater radioactivity was measured and pyrene  
127 was added as required in order to keep its concentration constant during the whole  
128 accumulation phase (24 h). Oysters were not fed during the exposure period. Four animals  
129 were collected at different times (0, 2, 5, 9, 19 and 24h) in order to follow the uptake kinetics  
130 of <sup>14</sup>C-pyrene and the formation of its metabolite. At the end of the exposure period (24h),  
131 seawater was sampled to detect whether metabolites could have been released by exposed  
132 animals.

133 At each sampling time, oyster soft tissues were dissected into five compartments: mantle,  
134 gills, muscle, digestive gland and remaining tissues (i.e., labial palps, gonad and heart). Each  
135 organ and tissue was weighed and crushed. Then, samples were treated with 200 µL of β-  
136 glucuronidase-aryl-sulfatase mixture containing 100 000 units mL<sup>-1</sup> of glucuronidase (Sigma)  
137 and 7500 units mL<sup>-1</sup> of sulfatase (Sigma) for enzymatic deconjugation. To this purpose, the

138 samples were buffered to pH 5 with 3 mL of 5 M potassium acetate buffer. They were then  
139 placed in an oven at 37°C during 20 hours.

140 Pyrene and 1-hydroxypyrene were extracted via two subsequent liquid/liquid extractions. The  
141 first extraction was performed by adding 5mL of a hexane/dichloromethane solution (50:50)  
142 and the second extraction was performed by adding 4mL of a methanol/dichloromethane  
143 solution (10:90) to extract pyrene and 1-hydroxypyrene, respectively. For both extractions,  
144 the samples were mechanically shaken for 30 minutes and then centrifuged at 6000 rpm for 5  
145 minutes. In the first extraction, the organic phase (5 mL) was recovered in a flat bottom flask.  
146 Following the second extraction, the organic phase was recovered and combined with the first  
147 one. Two mL of the extracted solution was radioanalysed to determine the amount of pyrene  
148 + 1-hydroxypyrene in each organ.

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

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

155

### 156 2.3.2. Depuration phase

157 At the end of the exposure period, the remaining organisms were placed in an open circuit  
158 50L seawater aquarium (salinity: 36±1 p.s.u.; temperature: 19 ±1 °C; pH: 8; light/dark cycle:  
159 12h/12h). At different times of the depuration period (0, 6, 18, 36, 96,192 and 336 h) four  
160 oysters were collected and their soft tissues dissected in order to follow the variation in  
161 pyrene and its metabolite. The dissected tissues and organs were processed according to the  
162 same method as previously described.



163 **2.4. Data analyses**

164 2.4.1. Uptake kinetics

165 A first order model was used to assess changes in pyrene concentration in oyster tissues along  
166 time during the exposure to waterborne pyrene. In this model the change in tissue activity  
167 with time was calculated by:

168

169 
$$dA_{\text{org}}/dt = k_u A_{\text{SW}} - k_e A_{\text{org}} \quad (\text{Eq. 1})$$

170

171 where  $A_{\text{org}}$  = activity of pyrene in tissue ( $\text{Bq g}^{-1}$  tissue)

172  $A_{\text{SW}}$  = activity of pyrene in seawater ( $\text{Bq g}^{-1}$  water)

173  $k_u$  = uptake rate constant ( $\text{Bq g}^{-1} \text{h}^{-1}$ )

174  $k_e$  = elimination rate constant ( $\text{Bq g}^{-1} \text{h}^{-1}$ )

175  $t$  = time (h)

176

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

179

180 
$$A_{\text{org}} t = A_{\text{SW}} (k_u / k_e) (1 - e^{-k_e t}) \quad (\text{Eq. 2})$$

181

182 where  $A_{\text{org}} t$  = tissue activity at time  $t$

183

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

186

187 
$$A_{\text{org}} / A_{\text{SW}} = \text{BCF} = k_u / k_e \quad (\text{Eq. 3})$$

188

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

191

#### 192 2.4.2. Depuration kinetics

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

195

$$196 \quad A_t = A_0 (e^{-k_e t}) \quad (\text{Eq. 4})$$

$$197 \quad A_t = A_{0s} (e^{-k_{es} t}) + A_{0l} (e^{-k_{el} t}) \quad (\text{Eq. 5})$$

198

199 where  $A_t$  = remaining activity at time  $t$  ( $\text{Bq g}^{-1}$ )

200  $A_0$  = activity at time  $0$  ( $\text{Bq g}^{-1}$ ), i.e. at the beginning of the depuration period

201  $k_e$  = depuration rate constant ( $\text{Bq g}^{-1} \text{h}^{-1}$ )

202

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

208

$$209 \quad T_{b1/2} = \ln 2 / k_e \quad (\text{Eq. 6})$$

210

211

212

### 213 2.4.3. Statistical analysis

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

219

## 220 **3. Results**

### 221 **3.1. Seawater exposure**

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

231 The final distribution of the contaminant in each compartment after 24h of exposure is  
232 presented in Fig. 2. Among tissues, gills contained half of the whole body burden  
233 radioactivity and consistently showed the highest rate of accumulation with a  $k_u$  of  $433 \text{ Bq g}^{-1}$   
234  $\text{h}^{-1}$  (Table 1). In contrast, the muscle was the organ with the lower rate of accumulation ( $k_u =$   
235  $38 \text{ Bq g}^{-1} \text{ h}^{-1}$ ) and thus, only contained a very low proportion (5%) of the total quantity of  $^{14}\text{C}$ -  
236 pyrene present in oyster soft tissues (Fig. 2).

237 Quantification of the pyrene metabolites was carried out at each sampling time, and the  
238 proportion of 1-hydroxypyrene represented between 4 and 14% of the total radioactivity (Fig.  
239 3).

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

242

### 243 **3.2. Depuration phase**

244 At the end of the exposure time, non-contaminating conditions were restored and depuration  
245 kinetics of the pyrene were followed in the organs and tissues of the oysters for 15d. The loss  
246 of incorporated <sup>14</sup>C-labelled pyrene followed a single or a double exponential model in the  
247 different body compartments (Fig. 4 and Table 2). In the muscle, gills and mantle, depuration  
248 was best described by a double-component exponential equation ( $R^2 = 0.42, 0.52$  and  $0.48$ ,  
249 respectively). The resulting biological half-lives ( $T_{b1/2}$ ) ranged from 1.17 (mantle) to 9.35  
250 hours (gills) for the short-lived compartment and from 2.75 (muscle) to 7.27 days (gills) for  
251 the long-lived compartment (Table 2). In contrast, a single-component exponential equation  
252 better fitted the depuration kinetics in the digestive gland and in the remaining tissues ( $R^2$   
253  $= 0.37$  and  $0.30$ , respectively). They were characterized by a relatively strong retention of  
254 pyrene: the resulting  $T_{b1/2}$  were 87.4 and 97.4 hours, respectively, (i.e., 3.64 and 4.05 days)  
255 (Table 2, Fig. 4).

256 The distribution of <sup>14</sup>C-labelled pyrene among the oyster soft tissues was determined at the  
257 end of the depuration period (Fig. 5). It differed from the distribution observed at the end of  
258 the exposure period (Fig. 2), with a lower fraction associated to the gills ( $24 \pm 10$  vs.  $47 \pm$   
259  $13\%$ ) and a higher fraction associated to the digestive gland ( $19 \pm 10$  vs.  $6 \pm 4\%$ ) and the  
260 remaining tissues ( $21 \pm 18$  vs.  $16 \pm 3\%$ ). At the end of the depuration period, the digestive  
261 gland displayed the highest pyrene activity (data not shown). In contrast, the gills had lost

262 50% of their activity during the depuration phase. Figure 5 also shows the distribution of 1-  
263 hydroxypyrene between the body compartments at the end of the depuration period. 1-  
264 hydroxypyrene was distributed in similar proportion as pyrene in the oyster body  
265 compartments.

266

#### 267 **4. Discussion**

268 Oysters accumulated very efficiently the  $^{14}\text{C}$ -labelled waterborne pyrene following a  
269 saturation model and after a short exposure period (24h), organisms reached the state of  
270 equilibrium. Among soft tissues, the gills accumulated rapidly and strongly the contaminant  
271 with a BCF of 2080 (Table 1) likely because of the high filtration rate of oysters which could  
272 be as high as  $3.9 \text{ L h}^{-1} \text{ g}^{-1}$  dry weight (Bougrier et al. 1995). Consequently, the gills displayed  
273 the highest activities at the end of the exposure phase. Absorption of pyrene onto gills might  
274 be facilitated in oysters, as it occurs in blue mussels *Mytilus edulis* which have a micellar  
275 layer which absorbs hydrocarbons (Lee et al. 1972a). Nevertheless, the accumulated pyrene  
276 was relatively rapidly lost from this tissue (Table 1). Indeed, pyrene proportion in the gills at  
277 the end of the depuration phase was half than at the end of the exposure period (Figs. 2 and  
278 5). This decrease is due to a fast depuration rate of pyrene in the gills (Table 1). At the same  
279 time, the increase of pyrene proportions in the digestive gland and remaining tissues suggest  
280 that it was transferred towards from the gills to these tissues as previously reported in other  
281 organisms (Neff, 1979). Therefore, the decrease of pyrene observed in the gills was also due  
282 to a relative increase of the activity in other compartments, such as the digestive gland (data  
283 not shown).

284 During the uptake phase, the mantle and the remaining tissues also accumulated pyrene but  
285 the steady-state was not reached for these compartments. This is probably due to the fact that,  
286 even if these tissues are in contact with seawater, their surface is much smaller than that of the

287 gills, and the cell types and the thickness of the epithelium differ completely (Auffret, 2003).  
288 The resulting uptake rates ( $k_u$ ) were lower for both tissues than for gills. During the  
289 depuration phase, the mantle released pyrene quickly. In contrast, the remaining tissues  
290 showed a slow increase of the pyrene proportion against the total pyrene content in oysters as  
291 well as of its metabolite proportion (against the total metabolite content in oysters) during the  
292 depuration period. It is well-known that tissues rich in lipids, e.g. gonads, accumulate  
293 preferentially PAHs because of the highly hydrophobic nature of the latter (Berthelin et al.  
294 2000, Meador et al. 1995). Moreover, a strong increase of the activity in the remaining tissues  
295 was noticed during the third sampling in the depuration period ( $t = 36h$ ). The four oysters  
296 studied at this moment showed the particularity of being in the reproduction stage and of  
297 having gonadic tissues more abundant than the average of other individuals. For this reason,  
298 having acknowledged the unlikely hypothesis that these observations could come partly from  
299 an error of manipulation, it seems that this increase of activity was rather due to a stronger  
300 retention in mature gonadic tissues. This conclusion is consistent with results of Ellis et al.  
301 (1993), where gonads from *C. virginica* displayed PAH concentrations five times higher than  
302 in somatic tissues.

303 In contrast, the digestive gland and the muscle which are not truly in direct contact with  
304 seawater displayed lower BCF than the gills, the mantle and the remaining tissues (Table 1).  
305 Nevertheless, the uptake rate for the digestive gland was much higher than the one of the  
306 muscle. The steady-state of pyrene in the digestive gland was reached very quickly, i.e. after  
307 9h while it took 19h in the muscle (Fig. 1). The results strongly suggest that during the  
308 exposure period pyrene was transferred from tissues in contact with seawater such as gills,  
309 towards the digestive gland. Moreover, the digestive gland showed a higher percentage of 1-  
310 hydroxypyrene (19%) in relation to total radioactivity (pyrene + 1-hydroxypyrene) than the  
311 other organs (9-12%) and the proportion of pyrene and its metabolite in the digestive gland

312 increased from 6-10% at the end of the accumulation period to 19-24% at the end of  
313 depuration period (Figs. 2 and 5). This suggests that the digestive gland had a stronger  
314 metabolism capacity of pyrene than the other tissues.

315 Among all the tissues studied, the digestive gland and the remaining tissues displayed the  
316 strongest retention capacity with  $T_{b/2}$  of 87.4 and 97.4h, respectively. Indeed, just like almost  
317 all organic contaminants, PAHs best store up in some tissues, namely in the hepatopancreas in  
318 invertebrates and in the liver in vertebrates (Meador et al. 1995). Because of their highly  
319 hydrophobic nature, PAHs are mostly accumulated in tissues with high lipid contents. The  
320 transformed PAH metabolites generally accumulate in the hepatopancreas (Lee et al. 1976;  
321 Neff et al. 1976; Meador et al. 1995). It has been proved that for some invertebrates and for  
322 several fish species exposed to PAHs either via water, food or sediments, the cytochrome  
323 P450 enzymatic system connected to an oxygenase function (MFO) is activated in their  
324 hepatic structure (Andersson and Forlin, 1992). In the case of fish, this enzymatic system  
325 allows excreting most of the PAHs bioaccumulated via the bile and the urine (Pritchard and  
326 Bend, 1991). This particular function was difficult to bring to light concerning bivalves. For a  
327 long time, it was admitted that bivalves did not possess a P450 system (Lee et al. 1972b;  
328 Vandermeulen and Penrose, 1978). However, more recent studies have shown that bivalves  
329 do possess a P450 system (Lake et al. 1985; McLeese and Burrige, 1987), allowing them to  
330 metabolize PAHs. As it is the case for the majority of marine invertebrates, such ability to  
331 metabolize PAHs is weaker than for vertebrates (Livingstone, 1994; Stegeman and Hahn,  
332 1994).

333 Data obtained on 1-hydroxypyrene in the tissues and organs of *C. gigas* seem to confirm that  
334 metabolism of pyrene in oysters is relatively weak and therefore would not be the driving  
335 mechanism responsible for its elimination. It is however possible that because of the large  
336 volumes of water they filter continuously, water-soluble metabolites would be rapidly

337 excreted and, therefore, not accumulated in the tissues (James, 1989). If the event lasts only  
338 for few days, it is very likely that most of the accumulated PAHs are going to be eliminated.  
339 In contrast, Meador et al. (1995) suggested that, during a chronic exposure, a non negligible  
340 fraction of the PAHs could be stored in lipids and become less subject to the elimination by  
341 diffusion or by metabolisation.

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

351

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358

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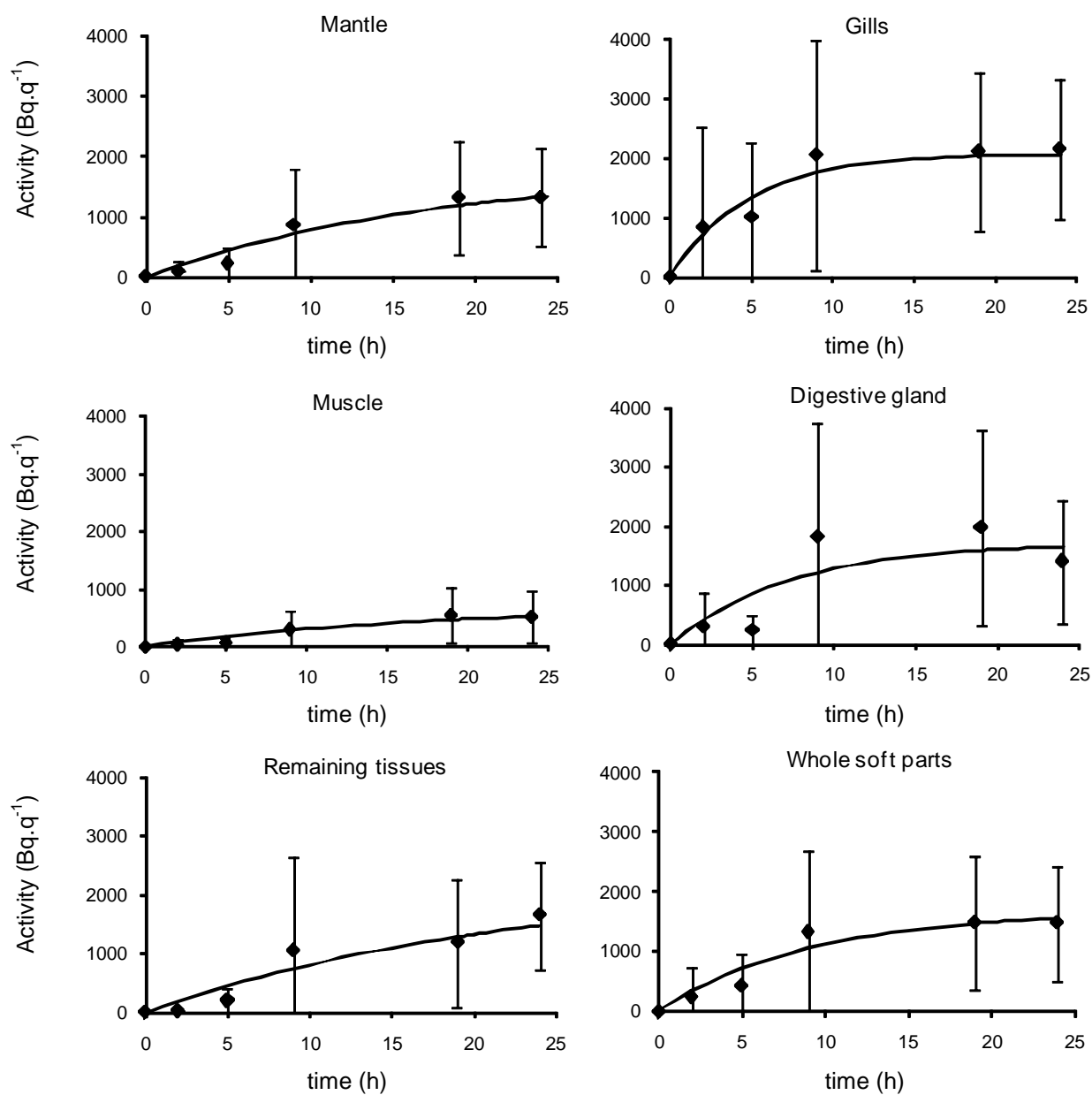


Figure 1: Uptake kinetics of  $^{14}\text{C}$ -labelled pyrene in five body compartments and in the whole soft parts of the oysters ( $n=4$ ) during the 24h seawater contamination period. Parameters and statistics of the uptake kinetics are given in Table 1.

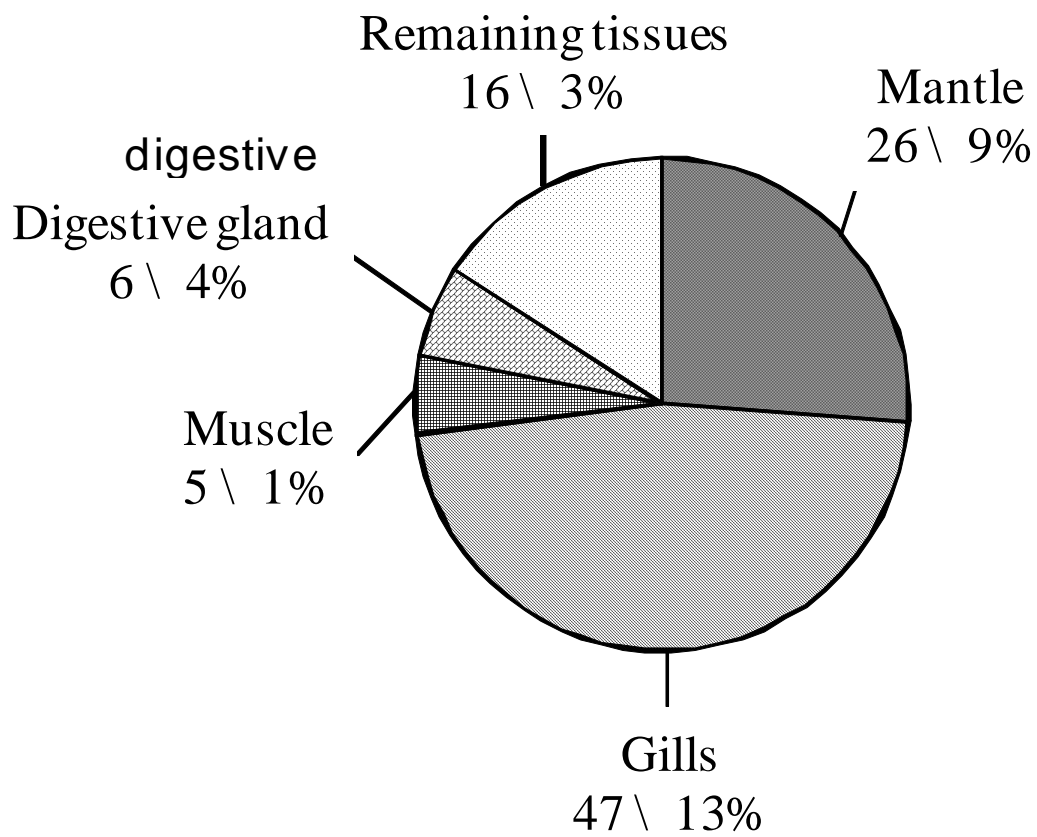


Figure 2: Distribution of <sup>14</sup>C-labelled pyrene (%) among the body compartments of oysters (n=4) at the end of the exposure period (24h).



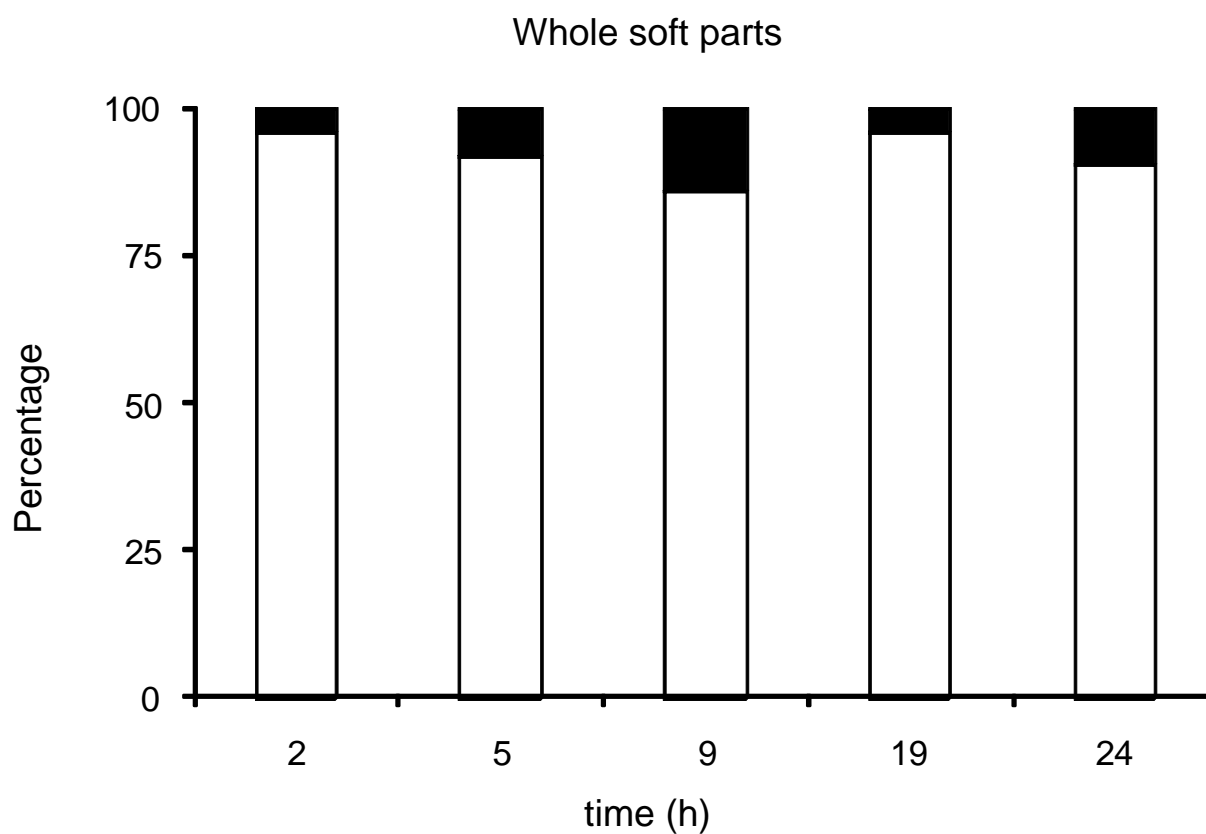


Figure 3: Proportion (%) between <sup>14</sup>C-labelled pyrene (in white) and 1-hydroxypyrene (in black) in the whole soft parts of the oysters (n=4) at the end of the exposure period (24h).

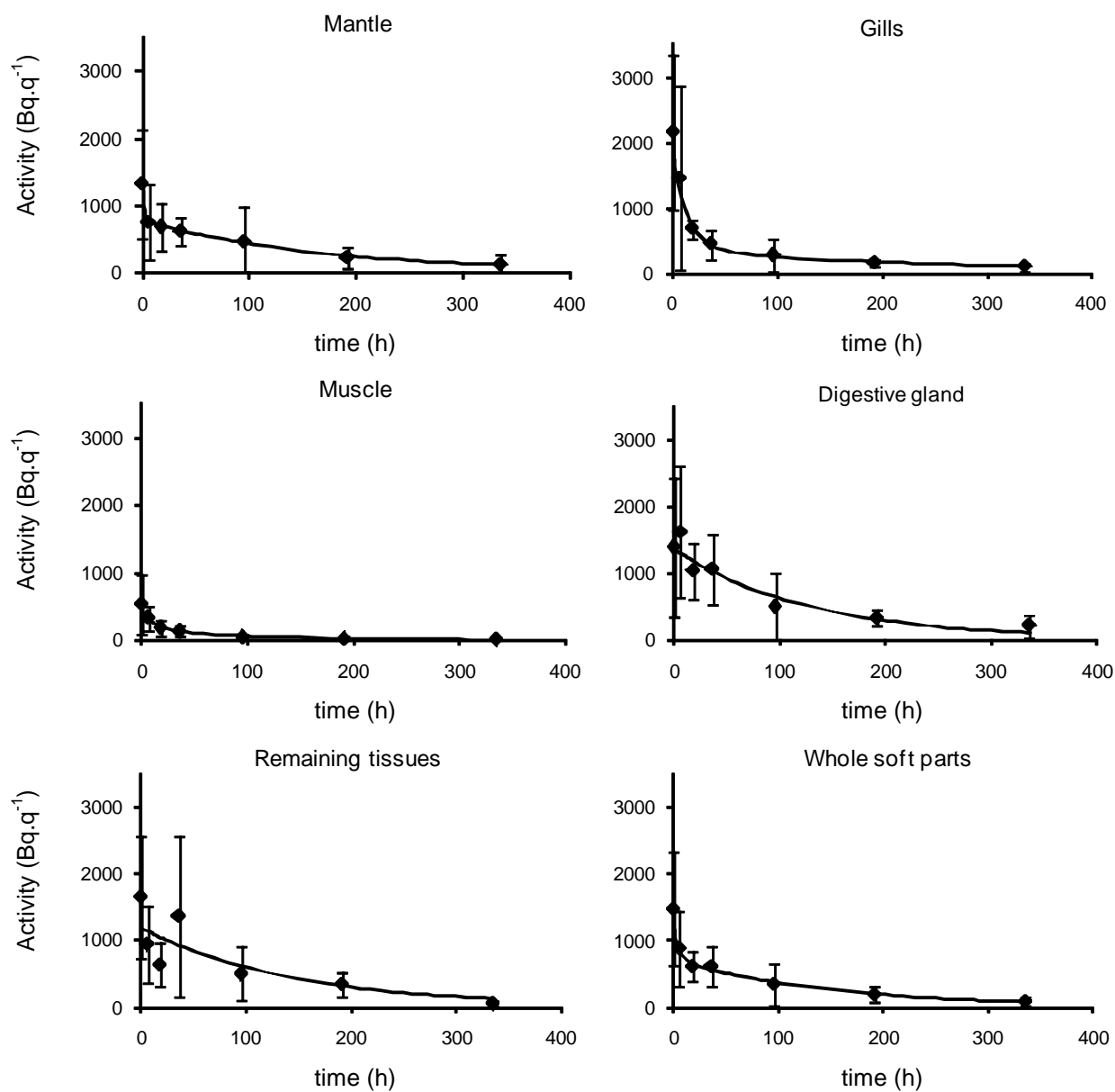


Figure 4: Loss kinetics of <sup>14</sup>C-labelled pyrene in five body compartments and in the whole soft parts of the oysters (n=4) after a 24h seawater exposure. Parameters and statistics of the loss kinetics are given in Table 2.

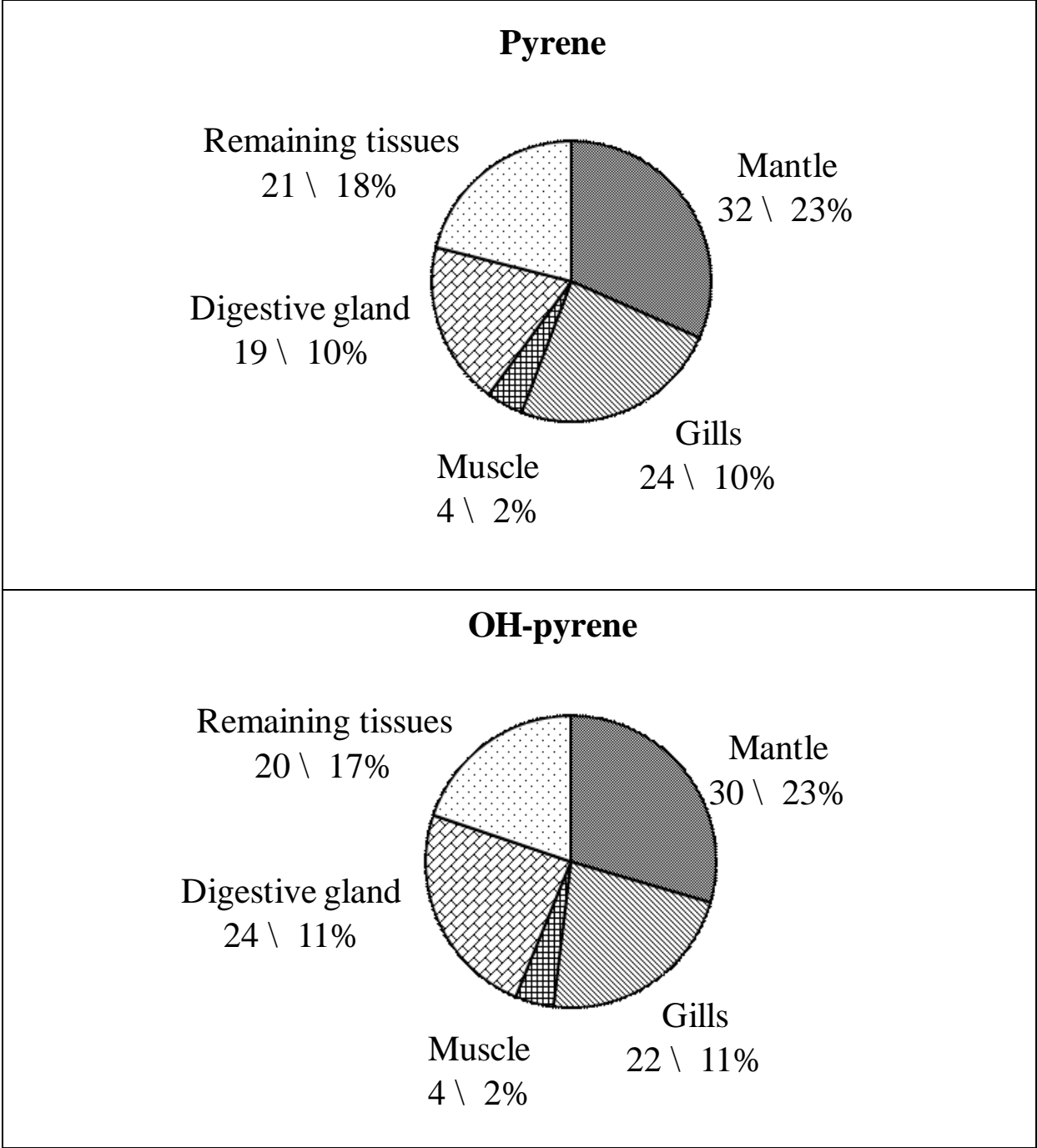


Figure 5: Distribution (%) of <sup>14</sup>C-labelled pyrene and 1-hydroxypyrene among the different body compartments of the oysters (n=4) at the end of the depuration period (15d).

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 24h seawater contamination ( $k_u$ =uptake rate constant;  $k_e$ = elimination rate constant; BCF=Bioconcentration factor)

Body compartment	$k_u$ (Bq g <sup>-1</sup> h <sup>-1</sup> )	$k_e$ (Bq g <sup>-1</sup> h <sup>-1</sup> )	BCF ( $k_u / k_e$ )	R <sup>2</sup>
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

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 24h 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;  $T_{b1/2}$ : 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