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

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► **To cite this version:**

Paco Bustamante, Andrea Luna-Acosta, Sarah Clamens, Roberto Cassi, H el ene Thomas-Guyon, et al.. Bioaccumulation and metabolisation of ¹⁴C-pyrene by the Pacific oyster *Crassostrea gigas* exposed via seawater. *Chemosphere*, 2012, 87 (8), pp.944-950. <10.1016/j.chemosphere.2012.01.049>. <hal-00682281>

HAL Id: hal-00682281

<https://hal.science/hal-00682281v1>

Submitted on 24 Mar 2012

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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 ¹⁴C-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 ¹⁴C-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 ± 1 p.s.u.; temperature: 19 ± 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⁻¹). Recorded mortality was
107 lower than 5% over the acclimation period.

108

109 **2.2. Radiotracer and radioanalyses**

110 The ¹⁴C-labelled 4, 5, 9, 10 pyrene was purchased from Sigma, USA. Specific activity was
111 $2.17 \cdot 10^9$ Bq mmol⁻¹. 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). ¹⁴C-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 ¹⁴C-labelled pyrene. The initial pyrene concentration in the aquarium was
125 0.27 µg L⁻¹. 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 ¹⁴C-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⁻¹ of glucuronidase (Sigma)
137 and 7500 units mL⁻¹ 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 upli-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_{org} = activity of pyrene in tissue (Bq g^{-1} tissue)

172 A_{SW} = activity of pyrene in seawater (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_{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 ¹⁴C-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 (Bq g^{-1})

200 A_0 = activity at time 0 (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}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 ± 630 (Table 1). Among the tissues, the gills accumulated rapidly and strongly
227 the contaminant with a BCF of 2080 ± 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 Bq g^{-1}
234 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}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 ¹⁴C-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 ¹⁴C-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 ± 10 vs. $47 \pm$
259 13%) and a higher fraction associated to the digestive gland (19 ± 10 vs. $6 \pm 4\%$) and the
260 remaining tissues (21 ± 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 ¹⁴C-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 L h⁻¹ 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

352 **5. Acknowledgments**

353 MW is an Honorary Senior Research Associate of the National Fund for Scientific Research
354 (NFSR, Belgium) and held a 2008-2009 Invited Expert position at LIENSs (CNRS-Université
355 de La Rochelle), supported by the Conseil Régional de Poitou-Charentes. This work was
356 supported by the IAEA and LIENSs. The IAEA is grateful for the support provided to its
357 Environment Laboratories by the Government of Monaco.

358

359 **6. References**

360 Andersson, T., Forlin, L., 1992. Regulation of the cytochrome P450 enzyme system in fish.
361 *Aquat. Toxicol.* 24, 1-20.

362 Ariese, F., Kok, S.J., Verkaik, M., Gooijer, C., Verlthorst, N.H., Hofstraat, J.W., 1993.
363 Synchronous fluorescence spectrometry of fish bile: A rapid screening method for the
364 biomonitoring of PAH exposure. *Aquat. Toxicol.* 26, 273-286.

365 Auffret, M. 2003. Atlas of histology and cytology of marine molluscs. Grizel H (ed.) Ifremer,
366 Plouzane France.

367 Axelman, J., Naes, K., Naf, C., Broman, D., 1999. Accumulation of polycyclic aromatic
368 hydrocarbons in semipermeable membrane device and caged mussels (*Mytilus edulis*) in
369 relation to water column phase distribution. *Environ. Toxicol. Chem.* 18, 2454-2461.

370 Baumard, P., 1997. Biogéochimie des composés aromatiques dans l'environnement marin.
371 Thèse d'Université Bordeaux I, 290p.

372 Baumard, P., Budzinski, H., Garrigues, P., Sorbe, J.C., Burgeot, T., Bellocq, J., 1998.
373 Concentrations of PAHs (Polycyclic Aromatic Hydrocarbons) in various marine organisms in
374 relation to those in sediments and to trophic level. *Mar. Pollut. Bull.* 36, 951-960.

375 Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J.F., Burgeot, T., Michel, X., Bellocq,
376 J., 1999. Polycyclic aromatic hydrocarbons (PAH) burden of mussels (*Mytilus* sp.) in different
377 marine environments in relation with PAH contamination, and bioavailability. *Mar. Environ.*
378 *Res.* 47, 415-439.

379 Beliaeff, B., O'Connor, T.P., Daskalakis, D.K., Smith, P.J., 1997. U.S. Mussel Watch data
380 from 1986 to 1994: Temporal trend detection at large spatial scales. *Environ. Sci. Technol.* 3,
381 1411-1415.

382 Berthelin, C., Kellner, K., Mathieu, M., 2000. Storage metabolism in the Pacific oyster
383 (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West Coast of
384 France). *Comp. Biochem. Physiol.* 125B, 359-369.

385 Bougrier, S., Geairon, P., Deslous-Paoli, J.M., Bacher, C., Jonquieres, G., 1995. Allometric
386 relationships and effects of temperature on clearance and oxygen consumption rates of
387 *Crassostrea gigas* (Thunberg). *Aquaculture* 134, 143-154.

388 Budzinski, H., Mazéas, O., Tronczynski, J., Désaunay, Y., Bocquené, G., Claireaux, G., 2004.
389 Link between exposure of fish (*Solea solea*) to PAHs and metabolites: Application to the
390 'Erika' oil spill. *Aquat. Living Resour.* 17, 329-334.

391 CNC 2004. Comité National de la Conchyliculture. Statistiques [on line] last accessed
392 February 2004. Available from World Wide Web:
393 <http://www.cncfrance.com/actualite/Default.htm>

394 Eisler, R., 1987. Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates:
395 a synoptic review. U.S. Fish and Wildlife Service Biological Report 85 (1.11).

396 Ellis, M.S., Choi, K.S., Wade, T.L., Powell, E.N., Jackson, T.J., Lewis, D.H., 1993. Sources
397 of local variations in polynuclear aromatic hydrocarbon and pesticides body burdens in
398 oysters (*Crassostrea virginica*) from Galveston bay, Texas. *Comp. Biochem. Physiol.* 100C,
399 689-698.

400 Escartin, E., Porte, C., 1999. Assessment of PAH Pollution in Coastal Areas from the NW
401 Mediterranean through the analysis of Fish Bile. *Mar. Pollut. Bull.* 38, 1200-1206.

402 Farrington, J.W., Tripp, B.W., 1993. International Mussel Watch. *Oceanus* 36, 62-64.

403 Fossi, M.C., Massi, A., Lari, L., Marsili, L., Focardi, S., Leonzio, C., Renzoni, A., 1995.
404 Interspecies differences in mixed function oxidase activity in birds: relationship between
405 feeding habits, detoxification activities and organochlorine accumulation. *Environ. Pollut.* 90,
406 15-24.

407 Giessing, A.M.B., Mayer, L.M., Forbes, T.L., 2003. Synchronous fluorescence spectrometry
408 of 1-hydroxypyrene: a rapid screening method for identification of PAH exposure in tissue
409 from marine polychaetes. *Mar. Environ. Res.* 56, 599-615.

410 Gunther, A.J., Davis, J.A., Hardin, D.D., Gold, J., Bell, D., Crick, J.R., Scelfos, G.M.,
411 Sericano, J., Stephenson, M., 1999. Long-term bioaccumulation monitoring with transplanted
412 bivalves in the San Francisco estuary. *Mar. Pollut. Bull.* 38, 170-181.

413 Hahn, M.E., 1998. The aryl hydrocarbon receptor: A comparative perspective. *Comp.*
414 *Biochem. Physiol.* 121C, 23-53.

415 Jaffé, R., Leal, I., Alvarado, J., Gardinalis, P., Sericano, J., 1995. Pollution effects of the Tuy
416 river on the Central Venezuelan Coast: Anthropogenic organic compounds and heavy metals
417 in *Tivela mactroidea*. *Mar. Pollut. Bull.* 30, 820-825.

418 James, M.O., 1989. Biotransformation and disposition of PAH in aquatic invertebrates. In:
419 Varanasi U. (Ed.). *Metabolism of polycyclic aromatic hydrocarbons in the aquatic*
420 *environment*. CRC Press, Boca Raton, FL: 69-91.

421 Kaag, N.H.B.M., Foekema, E.M., Scholten, M.C.Th., Van Straalen, N.M., 1997. Comparison
422 of contaminant accumulation in three species of marine invertebrates with different feeding
423 habits. *Environ. Toxicol. Chem.* 16, 837-842.

424 Krahn, M.M., Burrows, D.G., MacLeod, W.D., Malins, D.C., 1987. Determination of
425 individual metabolites of aromatic compounds in hydrolysed bile of English sole (*Parophrys*
426 *vetulus*) from Puget Sound, Washington. *Arch. Environ. Contam. Toxicol.* 16, 511-522.

427 Lake, J., Hoffman, G.L., Schimmel, S.C., 1985. Bioaccumulation of contaminants from Black
428 Rock Harbor dredged material by mussels and polychaetes. Tech. Rep. D-85-2, U.S.
429 Environmental Protection Agency, Washington, DC. 156p.

430 Lauenstein, G.G., 1995. Comparison of organic contaminants founding mussels and oysters
431 from a current Mussel Watch Project with those from archived mollusk samples of the 1970s.
432 *Mar. Pollut. Bull.* 30, 826-833.

433 Lee, R.F., Sauerheber, R., Benson, A.A., 1972a. Petroleum hydrocarbons: uptake and
434 discharge by the marine mussel *Mytilus edulis*. *Science* 177, 344-346.

435 Lee, R.F., Sauerheber, R., Dobbs, G.H., 1972b. Uptake, metabolism and discharge of
436 polycyclic aromatic hydrocarbons by marine fish. *Mar. Biol.* 37, 201-208.

437 Lee, R.F., Ryan, C., Neuhauser, M.L., 1976. Fate of petroleum hydrocarbons taken up from
438 food and water by the blue crab *Callinectes sapidus*. *Mar. Biol.* 37, 369-370.

439 Livingstone, D.R., 1994. Recent developments in marine invertebrates organic xenobiotic
440 metabolism. *Toxicol. Ecotoxicol. News* 1(3), 88-95.

441 McElroy, A., Leitch, K., Fay, A., 2000. A survey of in vivo benzo[a]pyrene metabolism in
442 small benthic marine invertebrates. *Mar. Environ. Res.* 50, 33-38.

443 McLeese, D.W., Burridge, L.E., 1987. Comparative accumulation of PAHs in four marine
444 invertebrates. In: Capuzzo J.M., Kesler D.R (Eds) *Oceanic Processes in Marine Pollution*.
445 Vol. I. Biological Processes and Wastes in the Oceans. Robert E. Krieger Publishing Co.,
446 Malabar, FL: 109-117.

447 Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U., 1995. Bioaccumulation of polycyclic
448 aromatic hydrocarbons by marine organisms. In: Wae GW (ed.) *Rev. Environ. Contam.*
449 *Toxicol.* 143, 79-166.

450 Neff, J.M., Cox, B.A., Dixit, D., Anderson, J.W., 1976. Accumulation and release of
451 petroleum-derived aromatic hydrocarbons by four species of marine animals. *Mar. Biol.* 38,
452 279-289.

453 Neff, J.M., 1979. Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fates
454 and biological effects. Applied science publishers Ltd. Ripples road, Barking, Essex, England,
455 262p.

456 Obana, H., Hori, S., Nakamura, A., Kashimoto, T., 1983. Uptake and release of polynuclear
457 aromatic hydrocarbons by short-necked clams (*Tapes japonica*). *Water Res.* 17, 1183-1187.

458 O'Connor, T.P., 1996. Trends in chemical concentrations in mussels and oysters collected
459 along the US coast from 1986 to 1993. *Mar. Environ. Res.* 41(2), 183-200.

460 O'Connor, T.P., Lauenstein, G.G., 2006. Trends in chemical concentrations in mussels and
461 oysters collected along the US coast: update to 2003. *Mar. Environ. Res.* 62(4), 261-285.

462 OSPAR (2010) OSPAR Coordinated Environmental Monitoring Programme (CEMP).
463 Agreement 2010–1. OSPAR Commission, 27p.

464 Palmork, K.H., Solbakken, J.E., 1981. Distribution and elimination of [9-¹⁴C]-phenanthrene
465 in the horse mussel (*Modiolus modiolus*). *Bull. Environ. Contam. Toxicol.* 26, 196-201.

466 Pereira, W.E., Hostettler, F.D., Rapp, J.B., 1992. Bioaccumulation of hydrocarbons derived
467 from terrestrial and anthropogenic sources in the Asian clam, *Potamocorbula amurensis*, in
468 San Francisco Bay Estuary. *Mar. Pollut. Bull.* 24, 103-109.

469 Phillips, D.J.H., 1976. The common mussel *Mytilus edulis* as an indicator of pollution by
470 zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged
471 by industry. *Mar. Biol.* 38, 71:80.

472 Pritchard, J.B., Bend, J.R., 1991. Relative roles of metabolism and renal excretory
473 mechanisms in xenobiotic elimination in fish. *Environ. Health Persp.* 90, 85-92.

474 ROCCH 2008. Résultats de la Surveillance de la Qualité du Milieu Marin Littoral, Edition
475 2008. Résultats acquis jusqu'en 2007. Ifremer/RST.LER/MPL/08.02/Laboratoire
476 environnement ressources de la Trinité-sur-Mer, 78 p.

477 Solé, M., Porte, C., Barcelo, D., Albaigés, J., 2000. Bivalves residue analysis for the
478 assessment of coastal pollution in the Ebro Delta (NW Mediterranean). *Mar. Pollut. Bull.* 40,
479 746-753.

480 Stegeman, J.J., Hahn, M;E., 1994. Biochemistry and molecular biology of monooxygenases:
481 current perspectives on forms, functions and regulation of cytochrome P450 in aquatic
482 species. In: Malins D.C. and Ostrander G.K. (Eds), *Aquatic Toxicology: Molecular,*
483 *Biochemical and Cellular Perspectives.* Lewis Publishers, Boca Raton, FL, pp 87-206.

484 Vandermeulen, J.H., Penrose, W.R., 1978. Absence of aryl hydrocarbon hydroxylase (AHH)
485 in three marine bivalves. *J. Fish Res. Board Can.* 35, 643-647.

486 Varanasi, U., Reichert, W.L., Stein, J.E., Brown, D.W., Sanborn, H.R., 1985. Bioavailability
487 and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment
488 from an urban estuary. *Environ. Sci. Technol.* 19, 836-841.

489 Varanasi, U., Stein, J.E., Nishimoto, M., 1989. Biotransformation and disposition of
490 polycyclic aromatic hydrocarbons (PAH) in fish. In: Varanasi U. (Ed.). *Metabolism of*
491 *polycyclic aromatic hydrocarbons in the aquatic environment.* CRC Press, Boca Raton, FL,
492 pp. 94-149.

493 Wade, T.L., Atlas, E.L., Brooks, J.M., Kennicutt, M.C., Fox, R.G., Sericano, J., Garcia-
494 Romero, B., DeFreitas, D., 1988. NOAA Gulf of Mexico status and trends program: trace
495 organic contaminant distribution in sediments and oysters. *Estuaries* 11, 171-179.

496 Watanabe, S., Shimada, T., Nakamura, S., Nishiyama, N., Yamashita, N., Tanabe, S.,
497 Tatsukawa, R., 1989. Specific profile of liver microsomal cytochrome P450 in dolphin and
498 whale. *Mar. Environ. Res.* 27, 51-65.

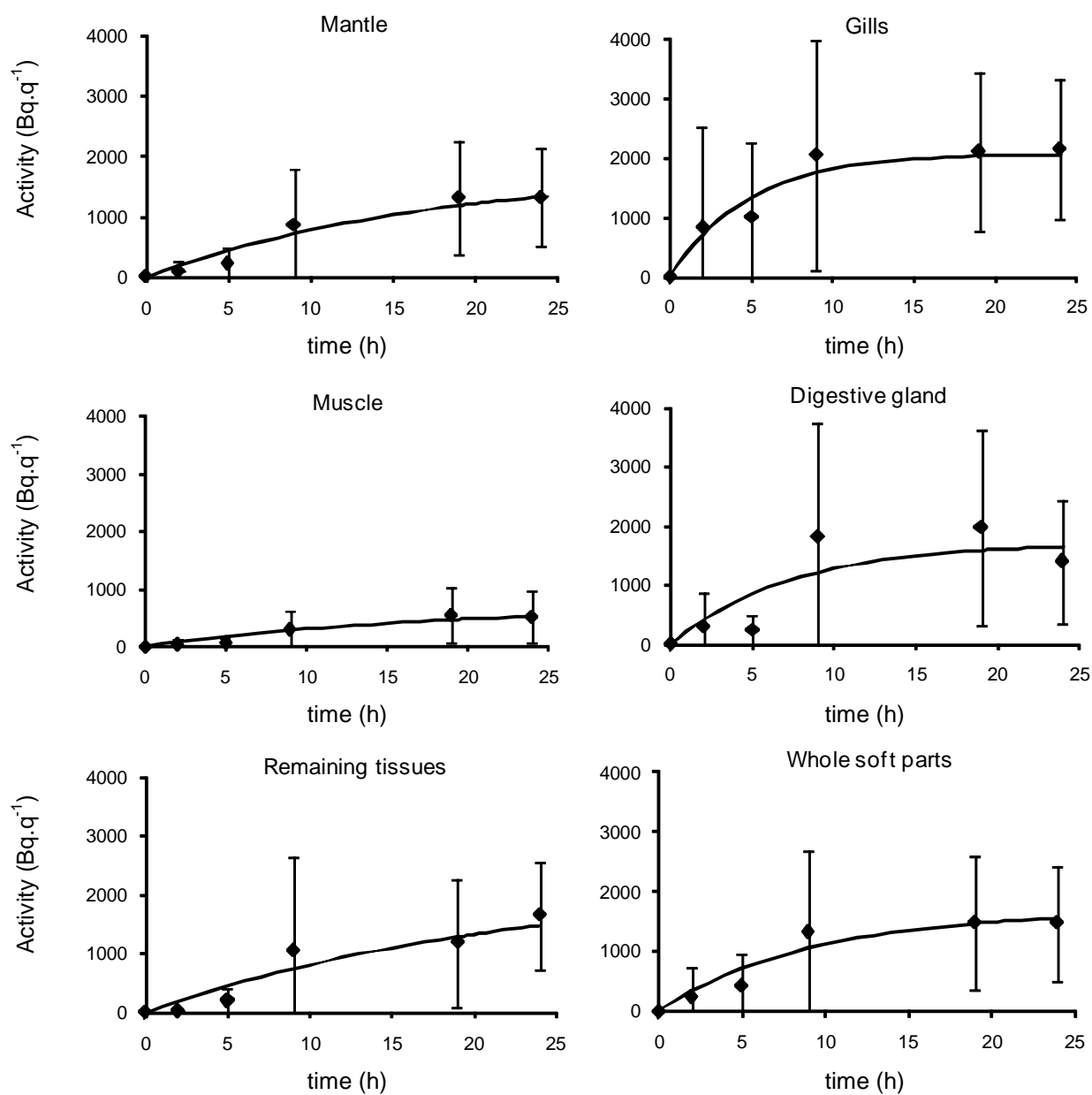


Figure 1: Uptake kinetics of ^{14}C -labelled pyrene in five body compartments and in the whole soft parts of the oysters ($n=4$) during the 24h seawater contamination period. Parameters and statistics of the uptake kinetics are given in Table 1.

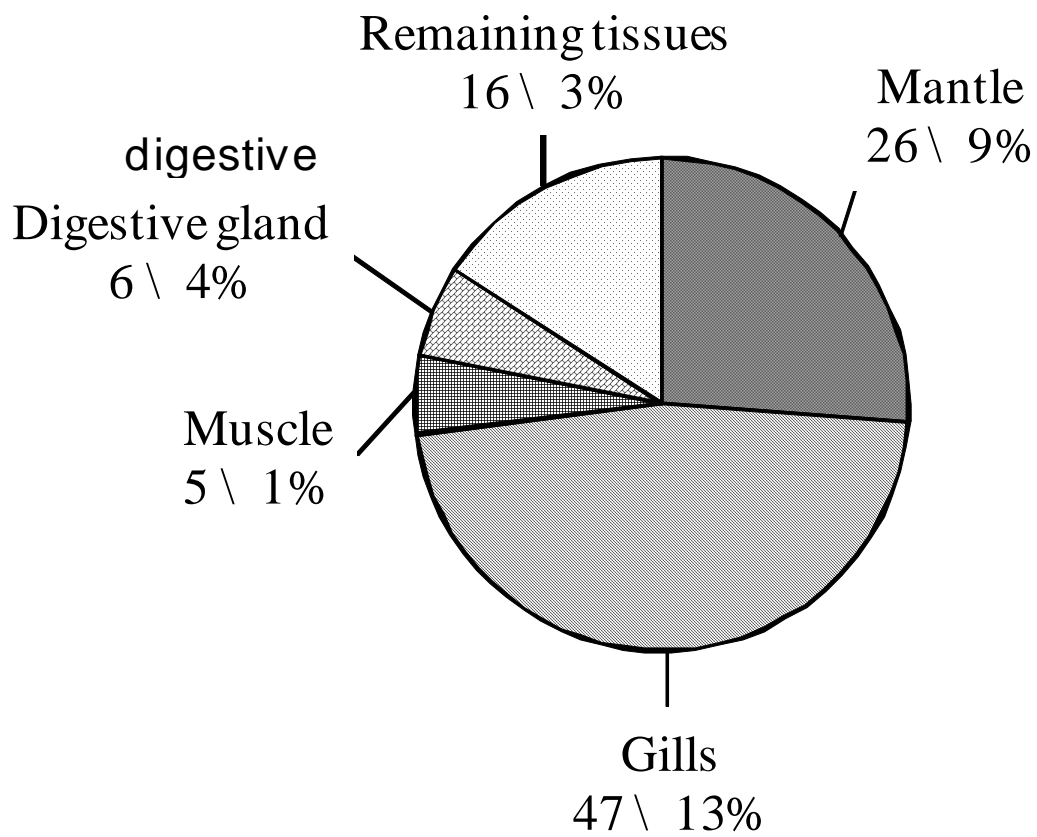


Figure 2: Distribution of ¹⁴C-labelled pyrene (%) among the body compartments of oysters (n=4) at the end of the exposure period (24h).

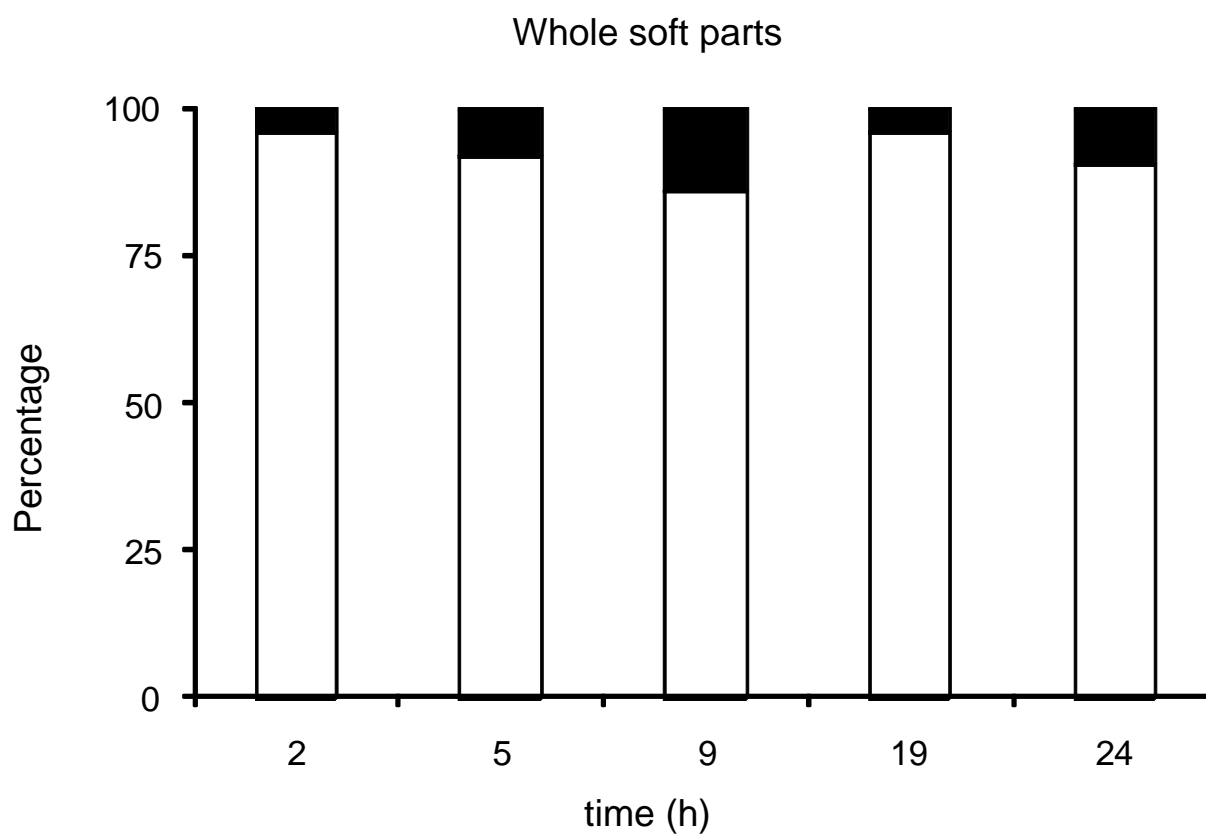


Figure 3: Proportion (%) between ¹⁴C-labelled pyrene (in white) and 1-hydroxypyrene (in black) in the whole soft parts of the oysters (n=4) at the end of the exposure period (24h).

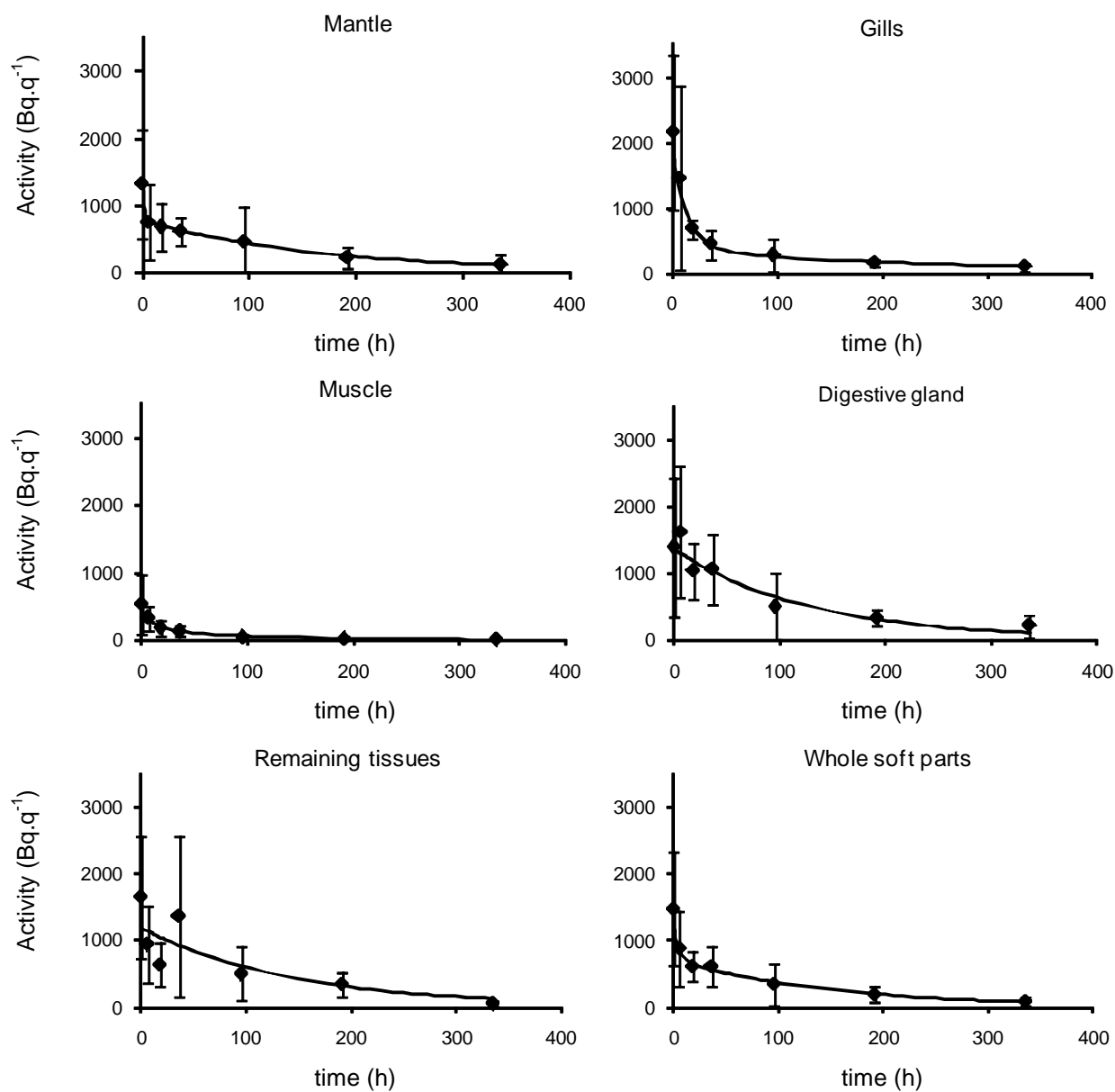


Figure 4: Loss kinetics of ^{14}C -labelled pyrene in five body compartments and in the whole soft parts of the oysters ($n=4$) after a 24h seawater exposure. Parameters and statistics of the loss kinetics are given in Table 2.

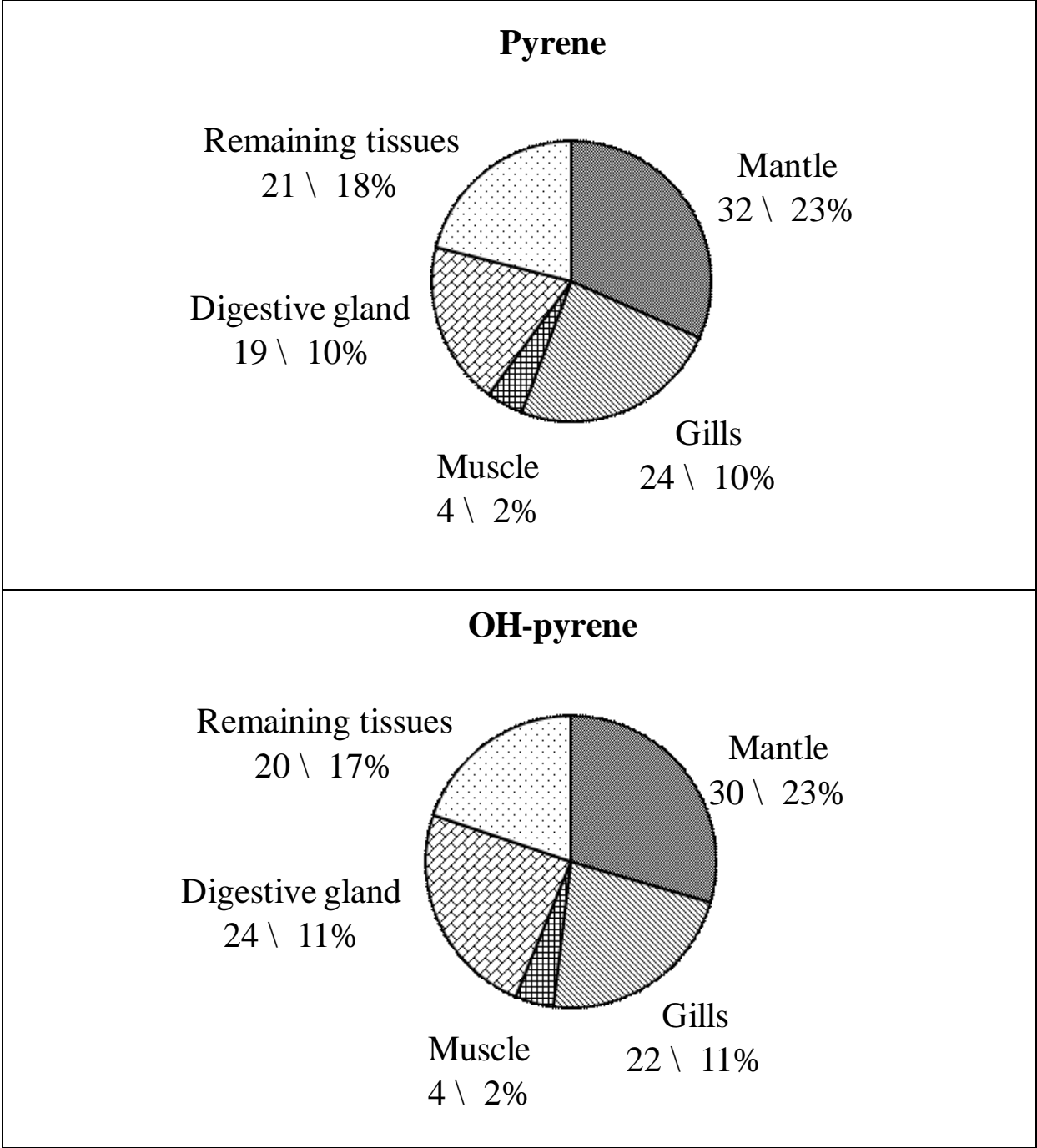


Figure 5: Distribution (%) of ¹⁴C-labelled pyrene and 1-hydroxypyrene among the different body compartments of the oysters (n=4) at the end of the depuration period (15d).

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 ⁻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

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