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## Assessment of the exposure pathway in the uptake and distribution of americium and cesium in cuttlefish (*Sepia officinalis*) at different stages of its life cycle

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### ► To cite this version:

Paco Bustamante, Jean-Louis Teyssié, Scott W. Fowler, Michel Warnau. Assessment of the exposure pathway in the uptake and distribution of americium and cesium in cuttlefish (*Sepia officinalis*) at different stages of its life cycle. *Journal of Experimental Marine Biology and Ecology*, 2006, 331 (2), pp.198-207. 10.1016/j.jembe.2005.10.018 . hal-00186647

**HAL Id: hal-00186647**

**<https://hal.science/hal-00186647>**

Submitted on 10 Nov 2007

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19 **ABSTRACT:** Laboratory radiotracer experiments were performed to study the uptake,  
20 assimilation and retention of americium ( $^{241}\text{Am}$ ) and cesium ( $^{134}\text{Cs}$ ) by the common cuttlefish  
21 *Sepia officinalis*. Uptake and loss kinetics of the radionuclides were measured following  
22 exposure through sediments, seawater and food at different stages of the animal's life cycle.  
23 Sediment was found to be a minor uptake pathway for both radionuclides in juveniles.  
24 Following a short seawater exposure, cuttlefish accumulated  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , but only to  
25 limited extent (whole-body CF < 2). Among the cuttlefish organs, branchial hearts and their  
26 appendages displayed the highest degree of uptake for  $^{241}\text{Am}$  (CF = 42 and 16, respectively),  
27 but these tissues contained low percentage of total  $^{241}\text{Am}$  due to their relatively small  
28 contribution to whole organism weight. The major fraction of incorporated radionuclides was  
29 associated with muscular tissues (viz. 65 and 82% of total  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively).  
30 Whole-body loss of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  was relatively rapid ( $T_{b1/2}$  = 14 and 6 d, respectively).  
31 After dietary exposure, around 60% and 30 % of ingested  $^{241}\text{Am}$  was assimilated into the  
32 tissues of juvenile and adult cuttlefish, respectively. However, assimilated  $^{241}\text{Am}$  was more  
33 strongly retained in adults than in juveniles ( $T_{b1/2}$  = 28 vs 5 d, respectively), suggesting that  
34 different mechanisms govern  $^{241}\text{Am}$  elimination at both ages. Ingested  $^{134}\text{Cs}$  was assimilated  
35 to a similar extent in juveniles (29%) and adults (23%), but the depuration rate was 4 times  
36 faster in adults. Our results strongly suggest that these two radionuclides followed different  
37 excretion pathways, and that the mechanisms can vary with age for a given radionuclide.

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39 Key words: Accumulation; Biokinetics; Cephalopods; Radionuclides; Retention

## INTRODUCTION

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Contamination of marine waters by radionuclides is a major concern in coastal areas which receive radioactive inputs from industries, accidents, and fallout from nuclear weapon testing. Surveys estimating concentrations of such radionuclides in water or sediments are often complemented by biomonitoring programs, and marine mussels have been used as biological monitors for radionuclides and heavy metals (Goldberg 1975, Goldberg et al 1978, Goldberg et al 1983, Goldberg & Bertine 2000). However, previous studies on the trophic transfer of trace elements and radionuclides have shown that herbivores such as mussels do not assimilate to any extent transuranic elements ingested with their food (e.g., Fowler 1982, Fisher et al. 1983, Warnau et al. 1996). Nevertheless this aspect has been little studied in higher trophic levels which are also used in many contaminant surveys. Therefore, there is a need to determine the bioaccumulation potential of marine carnivorous species for such elements.

Previous investigations with cephalopods have shown that these carnivorous species do bioaccumulate radionuclides in their tissues that can at times reach high levels (Suzuki et al. 1978, Guary et al. 1981, Yamada et al. 1999); however, little information is available on the pathways and rates of accumulation and retention of these radionuclides (Suzuki et al. 1978, Guary & Fowler 1982). Two long-lived radionuclides,  $^{241}\text{Am}$  and  $^{137}\text{Cs}$  (and  $^{134}\text{Cs}$ ), are present in fallout and also commonly found in nuclear wastes. The objective of our study was to examine the biokinetics of uptake and loss in cephalopods of these two contrasting radionuclides (particle-reactive americium and soluble cesium in sea water) in order to establish their bioaccumulation rates, tissue distribution and retention times as a function of (1) the uptake pathway, and (2) the life stage of the organism. The common cuttlefish *Sepia officinalis* was selected as a model species, and experimental exposures to these two

65 radionuclides via seawater, food and sediment were studied in both juvenile and adult  
66 individuals.

67

## 68 MATERIAL AND METHODS

69

### 70 Experimental organisms

71 Eggs of the common cuttlefish (*Sepia officinalis* L.) were obtained from cultured adults and  
72 were maintained in an aquarium with flowing seawater until hatching. Newly hatched  
73 juveniles (n = 25;  $0.387 \pm 0.071$  g wet wt) were selected and used in the experiments. Adult  
74 cuttlefish (n = 18;  $138 \pm 40$  g wet wt) were either reared in the Monaco Oceanographic  
75 Museum from hatching to one year old individuals, or collected by net fishing off Monaco (n  
76 = 5;  $253 \pm 97$  g wet wt). All organisms were subsequently maintained in filtered seawater in  
77 constantly aerated open circuit aquaria (salinity: 36 p.s.u.; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12/12 h  
78 dark/light cycle) until used in the radiotracer experiments.

79 Prior to experimentation, adults were anaesthetised in 2% ethanol in seawater for making  
80 biometric measurements, sex determination and for the insertion of a numbered plastic tag  
81 into the mantle fin to identify each animal during the experiments. In this way the same whole  
82 individual could be periodically radioanalyzed live in a small volume of sea water on the  
83 gamma well counter in order to reduce individual variability.

84

### 85 Radionuclides

86  $^{241}\text{Am}$  [ $t_{1/2} = 433$  yr] and  $^{134}\text{Cs}$  [ $t_{1/2} = 2$  yr] purchased from Amersham, UK, as nitrate and  
87 chloride salts, respectively, were used to trace americium and cesium biokinetics. The use of  
88  $^{134}\text{Cs}$  also served as an analogue for tracing the longer-lived  $^{137}\text{Cs}$  with a  $t_{1/2}$  of 33 yr. Stock  
89 solutions were prepared in their respective solutions (0.1 N) to obtain radioactivities which  
90 would allow using spikes of only a few microliters (typically 10 to 20  $\mu\text{L}$ ).

91

92 **<sup>241</sup>Am and <sup>134</sup>Cs uptake via sediments**

93 Sediments (2.5 kg dry wt) from the North Sea (Audresselles, Pas-de-Calais, France) were  
94 spiked for 4 d with <sup>241</sup>Am and <sup>134</sup>Cs tracer using the rolling jar method (Murdoch et al. 1997).  
95 Both <sup>241</sup>Am and <sup>134</sup>Cs are rapidly adsorbed onto sediments typically reaching near equilibrium  
96 after approximately 1 day (Carroll et al. 1997). Before initiating the experiment, radiolabelled  
97 sediments were held in flowing seawater overnight in order to leach weakly bound  
98 radiotracer. Sediments (50 g wet wt) were sampled at fixed intervals during the experiment to  
99 check for possible variations in radionuclide concentration. Juvenile cuttlefish (n = 9) were  
100 exposed for 29 d in a 20-L plastic aquarium containing 3 L of natural seawater running over a  
101 4 cm layer of spiked sediment. The level of sea water was maintained low in order to  
102 minimise the movements required for feeding and to maximise the contact time with  
103 sediments. During the experiment, all juvenile cuttlefish were fed twice daily with brine  
104 shrimp *Artemia salina* and were periodically  $\gamma$ -counted live in a well counter to follow the  
105 radionuclide uptake kinetics over the 29 d. At the end of the uptake experiment, 3 individuals  
106 were dissected to determine the distribution of the radionuclides among digestive gland,  
107 cuttlebone and remaining tissues (including other organs).

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109 **<sup>241</sup>Am and <sup>134</sup>Cs uptake via seawater and subsequent loss**

110 Newborn (n = 8) and adult (n = 5) cuttlefish were placed for 36 h and 8 h, respectively, in 70-  
111 L glass aquaria containing seawater spiked with <sup>241</sup>Am and <sup>134</sup>Cs (nominal activity: 6 kBq L<sup>-1</sup>  
112 each). Cuttlefish were then radioanalyzed and transferred to another 70-L aquarium supplied  
113 with natural flowing seawater. Juvenile cuttlefish were fed *A. salina* twice daily and were  
114 periodically  $\gamma$ -counted to follow radionuclide loss kinetics over 29 d. At the end of the loss  
115 period, 4 juveniles were dissected to determine the radionuclide distribution among digestive  
116 gland, cuttlebone and remaining tissues.

117 During the loss phase, adults were fed daily with soft parts of the mussel *Mytilus*  
118 *galloprovincialis*. Three adults were dissected after 8 h and the remaining two were dissected  
119 after 6 d of depuration. For each individual, the branchial heart appendages, branchial hearts,  
120 gills, digestive tract (after removal of the gut contents), genital tract, ovary or testes, ink sack,  
121 digestive gland, kidneys, mantle skin, mantle muscle, head and cuttlebone were separated,  
122 weighed, and their radionuclide content measured.

123

#### 124 **<sup>241</sup>Am and <sup>134</sup>Cs accumulation from food**

125 To prepare radiolabelled food, mussels (*M. galloprovincialis*) and brine shrimp (*A. salina*)  
126 were exposed for 7 d in plastic aquaria containing 4 L of natural seawater spiked with <sup>241</sup>Am  
127 and <sup>134</sup>Cs (nominal activity: 6 kBq L<sup>-1</sup> each). Radiolabelled seawater was renewed daily and  
128 the organisms were subsequently used as food for newborn (brine shrimp) and adult (mussels)  
129 cuttlefish.

130 For identification purposes, each individual juvenile cuttlefish (n = 8) was enclosed in a  
131 separate compartment allowing free circulation of seawater in a 70-L aquarium. After 1 h of  
132 ingesting radiolabelled brine shrimp, each individual was immediately  $\gamma$ -counted and replaced  
133 in the aquarium. From that time on cuttlefish were fed twice daily with non-contaminated *A.*  
134 *salina* and periodically  $\gamma$ -counted to determine radiotracer loss kinetics and assimilation  
135 efficiency. Throughout the depuration period (29 d), feces were removed 3 times per day to  
136 reduce possible indirect contamination by radiotracer recycling through leaching from the  
137 feces. At the end of the depuration period, 5 juveniles were dissected to determine the  
138 radiotracer distribution in their tissues.

139 Adult cuttlefish (n = 18) were held in a 3000 L aquarium and fed soft parts of the previously  
140 labelled mussels for 2 h. Immediately after ingestion, each individual was  $\gamma$ -counted and the  
141 same procedure was followed as for the juveniles. In addition, 3 adult cuttlefish were

142 dissected at each counting time to determine the radiotracer distribution among their organs  
143 and tissues.

144

#### 145 **Radioanalyses.**

146 Radioactivity was measured using a high-resolution  $\gamma$ -spectrometry system consisting of three  
147 coaxial Ge (N- or P-type) detectors (EGNC 33-195-R, Intertechnique) connected to a  
148 multichannel analyser and a computer with spectra analysis software (Interwinner,  
149 Intertechnique). The detectors were calibrated with appropriate standards for each of the  
150 counting geometries used, and measurements were corrected for background and physical  
151 decay of the radionuclides. Counting times were adapted to obtain relative propagated errors  
152 less than 5%. However, in a few cases, this counting precision could not be obtained even  
153 after 48 h of counting due to the very low activity in the extremely small dissected organs.  
154 Counting times ranged from 10 min to 1 h for whole cuttlefish, mussels and brine shrimp, and  
155 from 10 min to 48 h for the dissected organs and tissues. Following the relatively short  
156 counting periods in the containers of sea water, none of the organisms displayed any obvious  
157 abnormal or stressed behaviour when returned to their aquaria.

158

#### 159 **Data and statistical analyses.**

160 Uptake of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  from sediments and seawater was expressed, respectively, as  
161 whole-body transfer factors (TF) and concentration factors (CF) over time ( $\text{Bq g}^{-1}$  wet wt  
162 organism divided by the time-integrated  $\text{Bq g}^{-1}$  in sediments (TF) or seawater (CF).  
163 Radionuclide loss was expressed in terms of percentage of remaining radioactivity over time,  
164 i.e. radioactivity at time  $t$  divided by initial radioactivity measured in the organisms at the  
165 beginning of the depuration period. Loss kinetics were described either by a single-component  
166 exponential model:

167

$$A_t = A_0 e^{-k t},$$



168 where  $A_t$  and  $A_0$  are remaining activities (%) at time  $t$  (d) and 0, respectively, or by a 2-  
169 component exponential model:

$$170 \quad A_t = A_{0s} e^{-k_s t} + A_{0l} e^{-k_l t},$$

171 where the 's' subscript refers to a short-lived component (s component) and the 'l' subscript  
172 refers to a long-lived component (l component) (Whicker & Schultz 1982, Warnau et al.  
173 1996). The exponential model showing the best fit (based on calculation of the determination  
174 coefficients,  $R^2$ , and examination of the residuals) was selected.

175 The parameter  $k$  allows calculating of the radionuclide biological half-life (d) using the  
176 following equation:

$$177 \quad T_{b/2} = \ln 2/k.$$

178 Constants of the models and their statistics were estimated by iterative adjustment of the  
179 model and Hessian matrix computation, respectively, using the non-linear curve-fitting  
180 routines in the Systat 5.2.1 Software (Wilkinson 1988). Changes in radionuclide distribution  
181 among cuttlefish tissues and organs were tested for significance by the G procedure (adapted  
182 from the log-likelihood ratio test) for 2xk contingency tables (Zar 1996). Changes in % of  
183 radioactivity in a single tissue during the depuration period were tested by one-way ANOVA  
184 (after arcsin transformation of data) followed by the HSD Tukey's multiple comparison test.  
185 The significance level for statistical analyses was always set at  $\alpha = 0.05$ .

186

## 187 **RESULTS**

188

### 189 **Sediment exposure**

190 Regular measurements of  $^{241}\text{Am}$  concentration in sediment did not show any significant  
191 variation during the experimental time course ( $14.5 \pm 1.8 \text{ Bq g}^{-1}$  wet wt) while  $^{134}\text{Cs}$  activities  
192 decreased from  $12.4 \pm 0.1$  to  $7.0 \pm 0.4 \text{ Bq g}^{-1}$  wet wt.

193 Very low  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  activities were recorded in juveniles cuttlefish even after 29 d of  
194 exposure, and transfer factors (TF) were lower than 0.5 for both elements. Dissection of 3  
195 individuals after 29 d of exposure showed that for both radionuclides the digestive gland  
196 contained the highest proportion of the whole-body burden, i.e.  $47 \pm 28\%$  of  $^{241}\text{Am}$  and  $49 \pm$   
197  $12\%$  of  $^{134}\text{Cs}$  (Table 1).

198

### 199 **Seawater exposure**

200 Regular monitoring of the radionuclide concentrations in seawater allowed calculation of  
201 time-integrated radioactivities, viz.  $6.4 \pm 0.3$  and  $8.6 \pm 0.7$  kBq L<sup>-1</sup> for  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ ,  
202 respectively.

203 **Juveniles.** The whole-body activities measured after 36 h exposure in spiked seawater were  
204  $38 \pm 10$  and  $37 \pm 1$  Bq g<sup>-1</sup> wet wt for  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively, giving relatively low  
205 mean calculated whole-body CFs of  $6 \pm 2$  and  $4 \pm 1$  for these radionuclides.

206 Following transfer to non-contaminated seawater, loss kinetics of  $^{241}\text{Am}$  in juvenile cuttlefish  
207 were best fitted by a single-component exponential model whereas loss of  $^{134}\text{Cs}$  was best  
208 described by a two-component model (Figs 1A and 1B; Table 2). Loss kinetics were  
209 characterised by a biological half-life ( $T_{b/2}$ ) of 2 wk for  $^{241}\text{Am}$  and 1 wk for  $^{134}\text{Cs}$ .

210 At the end of the depuration period,  $^{134}\text{Cs}$  was mainly associated with the digestive gland of  
211 the young cuttlefish ( $61 \pm 4\%$  of whole-body activity) whereas  $^{241}\text{Am}$  was principally retained  
212 in the remaining tissues ( $61 \pm 13\%$ ) (Table 1). The lowest fraction of both radiotracers was  
213 found in the cuttlebone ( $< 15\%$  of the total activity).

214 **Adults.**  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  activities recorded in whole-body as well as in the different organs  
215 and tissues of adult cuttlefish after 8 h of exposure and corresponding CFs are presented in  
216 Table 3. The highest activities of  $^{241}\text{Am}$  were found in the branchial hearts and their  
217 appendages ( $264 \pm 85$  and  $103 \pm 66$  Bq g<sup>-1</sup> wet wt, respectively). In the case of  $^{134}\text{Cs}$ , the

218 branchial hearts, their appendages, gills and digestive tract displayed the highest activities,  
219 ranging from 9 to 13 Bq g<sup>-1</sup> wet wt.

220 When considering the tissue distribution of the radionuclides, muscle and skin of adults (i.e.  
221 the sum of the mantle muscles, skin and head) contained the highest proportion of <sup>241</sup>Am and  
222 <sup>134</sup>Cs, viz. 68 and 85%, respectively (Table 3). A somewhat lesser <sup>241</sup>Am fraction was found  
223 in the branchial hearts and digestive gland (10 ± 2% for both tissues). The radionuclide  
224 distribution among the tissues did not vary significantly (G test, p > 0.05) between the  
225 beginning and the end of the depuration period (Table 3).

226

### 227 **Food exposure**

228 In these experiments, juveniles (n = 8) were fed *ad libitum* radiolabelled adult brine shrimp  
229 for 1 h and adult cuttlefish (n = 18) ingested a total of 123 radiolabelled mussels during a 2-h  
230 feeding period. Immediately after feeding, all cuttlefish were γ-counted for determination of  
231 their radionuclide content.

232 **Juveniles.** The loss kinetics of ingested <sup>241</sup>Am and <sup>134</sup>Cs were best fitted by a 2-component  
233 exponential model composed of one rapid loss component followed by a single slow  
234 component (Figs 1C and 1D; Table 2). The short-lived component was derived from 40% and  
235 70% of the initially ingested <sup>241</sup>Am and <sup>134</sup>Cs activities, respectively (Table 2), and was  
236 characterised by a T<sub>b/2s</sub> < 1 d for both radionuclides. The long-lived component, which  
237 represents the fraction of the radionuclides actually absorbed by cuttlefish, displayed a T<sub>b/2l</sub> of  
238 5 d for <sup>241</sup>Am and 66 d for <sup>134</sup>Cs (Table 2). The same long-lived component allowed  
239 estimation of the assimilation efficiencies (AE) of the ingested nuclides. Results showed that  
240 <sup>241</sup>Am was readily assimilated in juveniles with an AE of 60% whereas the AE of <sup>134</sup>Cs was  
241 much lower, viz. 29% (Table 2). Dissections performed 29 d after feeding indicated that the  
242 highest proportion of remaining activity of both nuclides occurred in the digestive gland (ca.  
243 60% of the whole-body activity; Table 1).

244 **Adults.** The loss kinetics of both radionuclides ingested with food by adult cuttlefish were  
245 best described by a 2-component exponential model. As shown Figs 1E and 1F and in Table  
246 2, 69 and 78% of the ingested activity of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively, were rapidly lost with  
247 a  $T_{b/2s}$  of 4 and 13 h, respectively. The assimilated fraction of ingested  $^{241}\text{Am}$  was much lower  
248 in adults than in juveniles (AE = 31 vs 60%) but was lost at a slower rate from adults with a  
249  $T_{b/2l}$  of 28 d compared to 5 d in juveniles. For  $^{134}\text{Cs}$ , AEs were nearly similar for both age  
250 groups (AE = 23 vs 29% in adults and juveniles, respectively), however the radionuclide was  
251 depurated much faster in adults ( $T_{b/2l} = 16$  d) than in juveniles ( $T_{b/2l} = 66$  d).  
252 The tissue distribution of ingested radionuclides was determined on several occasions after  
253 feeding (Table 4). At the end of the depuration period, both  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  were  
254 predominantly distributed in the digestive gland (viz. 98 and 54%, respectively). The  
255 distribution of  $^{241}\text{Am}$  among tissues remained unchanged for 29 d of observation; in contrast,  
256 some significant changes were observed for  $^{134}\text{Cs}$  (G-test,  $p \leq 0.01$ ). For example, the  
257 proportion of  $^{134}\text{Cs}$  activity decreased in the muscular tissues (mantle muscles and head),  
258 whereas between 1 and 18 days of excretion it increased in the digestive gland (Table 4).

259

260

## DISCUSSION

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262 Cephalopods are an important resource of marine food and are fished and consumed in large  
263 quantities all around the world (Amaratunga 1983). Hence, the intake of contaminants such as  
264 radionuclides by humans through cephalopod consumption is a matter of potential concern.  
265 Cephalopods have been reported to concentrate natural and anthropogenic radionuclides such  
266 as  $^{210}\text{Po}$ ,  $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ , and  $^{239+240}\text{Pu}$  in their tissues (e.g. Smith et al. 1984, Finger & Smith  
267 1987, Yamada et al. 1999); however, little is known about the behaviour of radionuclides in  
268 these higher trophic level molluscs. To the best of our knowledge, only two species of  
269 cephalopods, viz. the octopus *Octopus vulgaris* and the squid *Doryteuthis bleekeri*, have been

270 used experimentally for investigating Am, Cs, and Pu biokinetics (Suzuki et al. 1978, Guary  
271 & Fowler 1982). These works were limited to measuring the uptake from seawater (i.e.  
272 Suzuki et al. 1978) or used a less than optimal experimental approach such as injecting the  
273 prey with radionuclides for the feeding experiments (Guary & Fowler 1982).

274 Cephalopods are found in a great variety of habitats from coastal waters to very deep ocean  
275 environments, with some living in direct contact with bottom sediments and others  
276 experiencing different environments during their life cycle (e.g. demersal species becoming  
277 temporarily pelagic during migration). Therefore, there is an obvious need to specifically  
278 determine 1) the uptake and retention of radionuclides at different stages of the life cycle of  
279 cephalopods, and 2) to assess the relative importance of the different pathways of exposure to  
280 radionuclides (sediments, seawater and food). In this context, the common cuttlefish *Sepia*  
281 *officinalis* appeared to be a good model cephalopod for such experiments as it spends part of  
282 its time buried in the sediment and is easy to rear and manipulate under laboratory conditions.

283

284 After a 1 month exposure to  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  through sediments, juvenile cuttlefish still  
285 exhibited very low transfer factors ( $\text{TF} < 0.5$ ), indicating that direct contamination due to  
286 burying into sediments is a minor uptake pathway for these radionuclides in cephalopods. The  
287 occurrence of a substantial fraction of both nuclides in internal tissues (viz. digestive gland  
288 and cuttlebone), which have no direct contact with the sediment, suggests that both  
289 radionuclides were progressively translocated from the tissues in direct contact with sediment  
290 and pore water to the digestive gland and, to a lesser extent, the cuttlebone (see Table 1). Such  
291 a translocation of elements to the cuttlebone was observed in a previous study on  
292 bioaccumulation of Cd in *S. officinalis* (Bustamante et al. 2002).

293 Following an acute contamination of adults via seawater, activities recorded in the whole  
294 cuttlefish suggest that they do not efficiently accumulate  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  directly from the  
295 dissolved phase. Indeed, both elements displayed low whole-body CFs ( $\text{CF} = 2$  for  $^{241}\text{Am}$  and

296 CF = 1 for  $^{134}\text{Cs}$ ). Nevertheless the 8 hr acute contamination time was relatively short and  
297 CFs are likely to be higher after a longer period of exposure. Activities of  $^{134}\text{Cs}$  measured in  
298 the different organs and tissues were all of the same order of magnitude. In contrast, for  
299  $^{241}\text{Am}$  the organs involved in respiration (the branchial hearts, their appendages and the gills)  
300 and digestion (digestive gland) displayed higher activities compared to other body  
301 compartments (see Table 3). However, in terms of their relative distribution in the whole  
302 body, both radionuclides were mainly located in muscular tissues which represent the main  
303 fraction (viz. 75%) of the total body weight: muscles and head contained 65% and 82% of the  
304 total  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively. A longer exposure (14 d) of *Octopus vulgaris* to  $^{137}\text{Cs}$  in  
305 sea water gave a similar distribution (i.e. 88%) of the radioisotope in the edible parts (Suzuki  
306 et al. 1978). In contrast, a 15-d exposure of the same species in seawater spiked with  $^{241}\text{Am}$   
307 resulted in only ca. 20% of the retained radioactivity being found in the muscular parts with  
308 most of the  $^{241}\text{Am}$  concentrated in the branchial hearts and their appendages (Guary & Fowler  
309 1982). In our experiments with *S. officinalis*, these tissues contained low percentages of the  
310 total  $^{241}\text{Am}$ , most probably because of the short duration of the exposure in sea water.  
311 Nevertheless, even after 8 hours they significantly concentrated the radionuclide with CFs as  
312 high as 42 in the branchial hearts and 16 in the appendages.

313 Both field and laboratory investigations with cephalopods have demonstrated the ability of  
314 branchial hearts to concentrate transuranic elements to fairly high levels (Guary et al. 1981,  
315 Guary & Fowler 1982). This ability could be related to the presence of polyhedral cells  
316 containing granular, Fe-rich, pigment concretions (adenochromes) (e.g., Fox & Updegraff  
317 1943, Nardi & Steinberg 1974). The affinity of  $^{241}\text{Am}$  for adenochromes in the branchial  
318 hearts has been demonstrated using autoradiographic techniques (Miramand & Guary  
319 1981); however, adenochromes have not been found in the appendages of the branchial hearts  
320 (Nardi & Steinberg 1974), an observation which suggests that they serve as an excretion  
321 pathway for  $^{241}\text{Am}$  rather than as storage sites.

322 Following exposure of juveniles in contaminated seawater, subsequent  $^{241}\text{Am}$  and  $^{134}\text{Cs}$   
323 elimination over a one month period displayed a one- and a two-component exponential loss  
324 model, respectively. Whole-body loss was relatively rapid for both nuclides with a mean  $T_{b1/2}$   
325 of 14 and 6 d, respectively. After 29 d of depuration, residual  $^{241}\text{Am}$  was mainly located in the  
326 remaining tissues (comprising the branchial hearts) of juveniles. However, as the juvenile  
327 branchial heart was not fully developed, additional work is needed to examine its role as a  
328 preferential storage organ such as occurs in adults.

329 In the case of dietary exposure,  $31 \pm 3\%$  of the ingested  $^{241}\text{Am}$  was assimilated into the tissues  
330 of adult cuttlefish, whereas in contrast  $^{241}\text{Am}$  was absorbed to a much greater extent in  
331 juveniles ( $\text{AE} = 60 \pm 10\%$ ). This difference between AEs could be due to differences in  
332 efficiency of digestion between juveniles and adults, since digestive metabolism is thought to  
333 decrease with age in cephalopods (Mangold 1989). More likely, however, the difference could  
334 also be partly due to variations in the bioavailability of  $^{241}\text{Am}$  in the food used for juveniles  
335 (brine shrimp) compared to that ingested by adults (i.e. mussels). Indeed, different storage  
336 mechanisms in prey can determine metal bioavailability to higher trophic levels (Wallace &  
337 Lopez 1997, Wallace & Luoma 2003, Seebaugh et al. 2005), which in a similar fashion could  
338 lead to different proportions of transferable  $^{241}\text{Am}$ . Overall, such very high AEs for  $^{241}\text{Am}$  in  
339 the common cuttlefish are rather unique whereas in herbivorous bivalves, many crustaceans,  
340 echinoids, and fish, assimilation of particle-reactive transuranic elements is typically very low  
341 (e.g. Fowler et al., 1976; Pentreath 1977, 1981; Fisher et al., 1983; Carvalho and Fowler,  
342 1985; Warnau et al., 1996). Such a difference could be related to the organism's feeding  
343 regime since cephalopods are strict carnivores. For instance, unexpected high AEs (up to  
344 60%) of plutonium have also been found in carnivorous crustaceans, *viz.* the crabs *Carcinus*  
345 *maenas* and *Cancer pagurus* (Fowler and Guary, 1977). Hence, the contribution of the  
346 radionuclide from the trophic pathway is very likely to be strongly enhanced in certain  
347 carnivorous invertebrates.

348 Once assimilated,  $^{241}\text{Am}$  was retained to a much greater degree in adults, with a half-life  
349 approximately 6 times longer than in juveniles (i.e. 28 d vs 5 d), which suggests that different  
350 processes govern  $^{241}\text{Am}$  elimination/retention in the two age groups. In other molluscs such as  
351 mussels,  $^{241}\text{Am}$  has been reported to be strongly retained in the digestive gland (Bjerregaard et  
352 al. 1985, Fisher & Teyssié 1986), a finding which is in agreement with our own observations.  
353 Indeed, after 29 d of depuration, the major fraction of residual  $^{241}\text{Am}$  was in the digestive  
354 gland, with a much higher fraction in adults than in juvenile cuttlefish (98% vs 59%). In the  
355 digestive gland of *Octopus vulgaris*, Guary & Fowler (1982) reported that  $^{241}\text{Am}$  is likely  
356 associated with the cellular waste products such as brown bodies. Considering this hypothesis  
357 together with our experimental observations, the longer retention of  $^{241}\text{Am}$  observed in adult  
358 *S. officinalis* could be due to a more rapid turnover of digestive cells in juveniles, thus  
359 resulting in a higher  $^{241}\text{Am}$  excretion rate.

360 In contrast to  $^{241}\text{Am}$ , ingested  $^{134}\text{Cs}$  was assimilated to a similar extent in juveniles (29%) and  
361 adults (23%), and the depuration rate constant was 4 times higher in adults resulting in a  
362 significantly much shorter  $^{134}\text{Cs}$  half-life in adults (16 d) than in juveniles (66 d) (Table 2).  
363 The longer retention time of  $^{134}\text{Cs}$  in juveniles is difficult to explain since, for certain  
364 transition elements (Ag, Cd, Co and Zn) previously investigated in cuttlefish (Bustamante et  
365 al. 2002, 2004) as well for  $^{241}\text{Am}$  (our study), early juveniles displayed shorter retention half-  
366 times than adults. The main difference in tissue distribution of  $^{134}\text{Cs}$  between adults and  
367 juveniles was the higher proportion present in the cuttlebone ( $22 \pm 21\%$  in juveniles vs  $2 \pm 0\%$   
368 in adults; see Tables 1 and 4). This higher skeleton-associated fraction is most likely tightly  
369 bound and hence results in the high retention half-time observed. Although, our results clearly  
370 indicate that  $^{134}\text{Cs}$  does not follow the same excretion pathway as  $^{241}\text{Am}$ , the above  
371 interpretation should be considered with caution since to the best of our knowledge,  
372 calcareous skeletons have not been shown to act as a particularly efficient sink for cesium in  
373 contrast with other elements such as, e.g.,  $^{241}\text{Am}$  or Pb (see e.g. Grillo et al. 1981, Warnau et



374 al. 1998). Furthermore, in our feeding experiment the very low activities measured in minute  
375 organs such as juvenile cuttlebone were frequently associated with low counting accuracy,  
376 which in turn can lead to a rather poor estimation of radioactivities and hence radionuclide  
377 distribution (as indicated by the elevated SD value of the cuttlebone-associated fraction of  
378  $^{134}\text{Cs}$ ). Clearly, further study is needed to better understand the observed differences in the  
379 fate of  $^{134}\text{Cs}$  and  $^{241}\text{Am}$  once taken up in young and adult cephalopod tissues.

380

381 **Acknowledgements.** We thank N. Tevenin and P. Gilles (Musée Océanographique, Monaco)  
382 for providing us with the organisms. We are also grateful to E. Boucaud-Camou (Université  
383 de Caen, France) for her advice on cuttlefish rearing. MW is an Honorary Research Associate  
384 of the National Fund for Scientific Research (NFSR, Belgium). The International Atomic  
385 Energy Agency is grateful for the support provided to its Marine Environment Laboratory by  
386 the Government of the Principality of Monaco.

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513

Captions to Figure

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515

516 Fig. 1. *Sepia officinalis*. Whole-body loss kinetics of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  (% of remaining  
517 activity; mean  $\pm$  SD):

518 (A, B) juvenile cuttlefish previously exposed to spiked seawater for 36 h (n = 8 from day 0 to  
519 20 and n = 4 on day 29);

520 (C, D) juvenile cuttlefish previously fed radiolabelled brine shrimp (n = 8 from day 0 to 22  
521 and n = 5 on day 29);

522 (E, F) adult cuttlefish previously fed radiolabelled mussels (n = 18 on day 0, n = 15 from day  
523 1 to 18, n = 12 from day 19 to 29).

524 Parameters for the best fitting equations are given in Table 3.

525 **Table 1.** *Sepia officinalis*. Distribution (%; mean  $\pm$  SD) of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  among three body  
 526 compartments of juvenile cuttlefish (1) after a 29-d exposure to spiked sediments, (2) after a  
 527 29-d depuration following a 36-h exposure to spiked seawater, and (3) after a 29-d depuration  
 528 following ingestion of spiked food (brine shrimp).

529

Exposure pathway	N	Body compartment		
		Digestive gland	Cuttlebone	Remaining tissues
<b>1. Sediments (29-d exposure)</b>	3			
$^{241}\text{Am}$		49 $\pm$ 12	12 $\pm$ 3	39 $\pm$ 15
$^{134}\text{Cs}$		47 $\pm$ 28	17 $\pm$ 4	36 $\pm$ 24
<b>2. Seawater (29-d depuration)</b>	4			
$^{241}\text{Am}$		27 $\pm$ 13	13 $\pm$ 0	61 $\pm$ 13
$^{134}\text{Cs}$		61 $\pm$ 4	5 $\pm$ 0	34 $\pm$ 4
<b>3. Feeding (29-d depuration)</b>	5			
$^{241}\text{Am}$		59 $\pm$ 23	12 $\pm$ 10	29 $\pm$ 16
$^{134}\text{Cs}$		60 $\pm$ 27	22 $\pm$ 21	18 $\pm$ 14

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**Table 2.** *Sepia officinalis*. Parameters of the equations best fitting the whole-body loss kinetics of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  in cuttlefish previously exposed to the radionuclides via different pathways: (1) juveniles previously exposed for 36 h via seawater; (2) juveniles previously fed radiolabelled brine shrimp; (3) adults previously fed radiolabelled mussels. O and T: one- and two-exponential loss equations, respectively;  $A_{oi}$ : assimilation efficiency (AE); ASE: asymptotic standard error;  $R^2$ : determination coefficient; p: probability of the model adjustment.

Pathway	Model	$A_{os}$ (ASE)	$\lambda_s$ (ASE)	$T_{b1/2s}$ (days)	$A_{oi}$ (ASE)	$\lambda_l$ (ASE)	$T_{b1/2l}$ (days)	$R^2$	p
<b>1. Loss in juveniles after seawater exposure</b>									
$^{241}\text{Am}$	O	87.7 (2.8)	0.048 (0.005)	14	-	-	-	0.96	< 0.001
$^{134}\text{Cs}$	T	74.6 (7.1)	1.015 (0.163)	0.7	25.6 (6.8)	0.114 (0.036)	6.1	0.97	< 0.001
<b>2. Loss in juveniles after a single feeding on brine shrimp</b>									
$^{241}\text{Am}$	T	39.6 (10.5)	1.282 (0.654)	0.5	60.3 (10.1)	0.137 (0.029)	5.1	0.95	< 0.001
$^{134}\text{Cs}$	T	70.3 (4.4)	0.972 (0.153)	0.7	29.2 (3.6)	0.011 (0.008)	66	0.98	< 0.001
<b>3. Loss in adults after a single feeding on mussels</b>									
$^{241}\text{Am}$	T	68.6 (3.8)	4.125 (3.683)	0.17	31.4 (2.5)	0.025 (0.009)	28	0.95	< 0.001
$^{134}\text{Cs}$	T	77.6 (4.4)	1.310 (0.197)	0.53	22.5 (3.7)	0.045 (0.019)	16	0.95	< 0.001



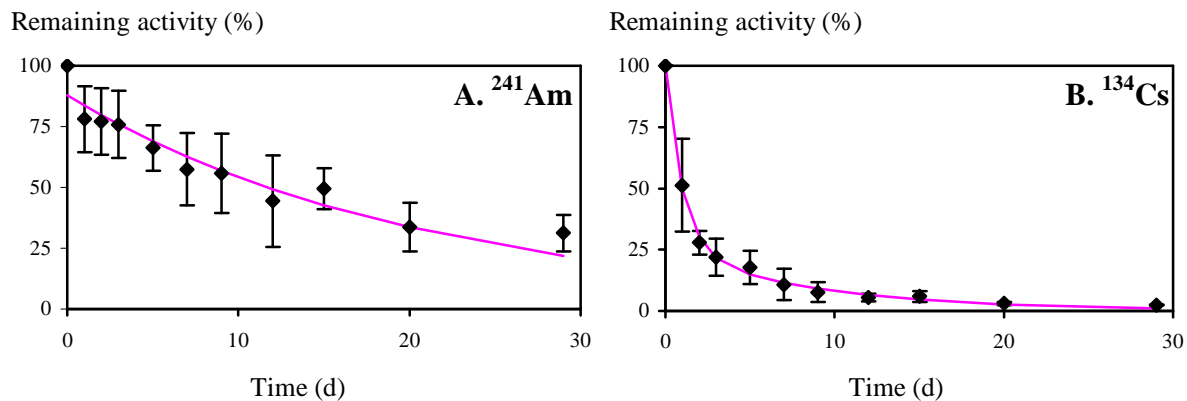
**Table 3.** *Sepia officinalis*. Concentration factors (CFs, mean), radionuclide activities (Bq g<sup>-1</sup> wet wt; mean ± SD) and tissue distribution of radioactivity (%; mean ± SD) in adult cuttlefish after 8 h of exposure via seawater (n = 3) and after 6 d of depuration (n = 2).

Tissue	% wet wt	<sup>241</sup> Am					<sup>134</sup> Cs				
		Accumulation (8 h)			Depuration (6 d)		Accumulation (8 h)			Depuration (6 d)	
		CF	Activity	%	Activity	%	CF	Activity	%	Activity	%
Branchial heart appendages	0.03 ± 0.004	16	103 ± 66	< 1	56	< 1	1	9 ± 2	< 1	1	< 1
Branchial hearts	0.10 ± 0.02	42	264 ± 85	3 ± 0	203	3	2	13 ± 1	< 1	2	< 1
Gills	2.3 ± 0.3	7	42 ± 14	10 ± 2	11	4	1	10 ± 2	4 ± 0	2	2
Digestive tract	2.6 ± 0.6	2	15 ± 5	4 ± 2	4	2	1	10 ± 1	4 ± 1	1	1
Genital tract	3.6 ± 1.0	1	9 ± 5	3 ± 1	2	1	< 1	4 ± 1	2 ± 0	< 1	4
Ink sack	0.6 ± 0.2	2	12 ± 1	1 ± 0	7	1	1	7 ± 3	1 ± 0	2	< 1
Skin	6.4 ± 2.1	1	6 ± 4	3 ± 1	3	2	< 1	4 ± 2	3 ± 0	< 1	4
Digestive gland	4.3 ± 1.2	3	22 ± 16	10 ± 2	28	11	< 1	3 ± 2	2 ± 1	1	1
Kidney	0.07 ± 0.07	2	13 ± 5	< 1	4	< 1	1	8 ± 5	< 1	1	< 1
Muscle	35 ± 2	1	7 ± 2	26 ± 4	10	52	1	6 ± 1	36 ± 3	2	55
Head	40 ± 1	1	9 ± 3	39 ± 1	4	23	1	7 ± 2	46 ± 3	1	32
Cuttlebone	5.1 ± 0.6	< 1	2 ± 1	1 ± 1	2	1	< 1	1 ± 1	1 ± 1	< 1	< 1
Whole cephalopod	100	2	10 ± 3	100	11	100	1	6 ± 2	100	3	100

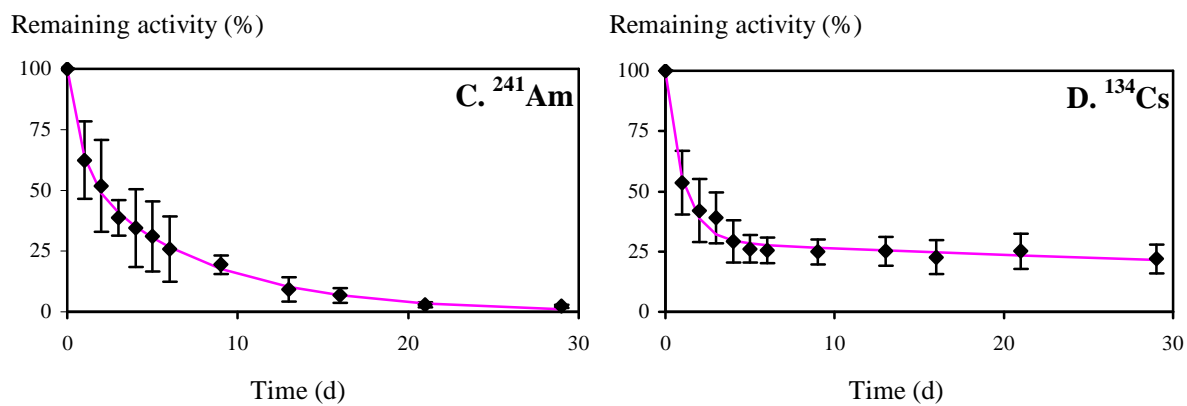
**Table 4.** *Sepia officinalis*. Radionuclide distribution among tissues (%; mean  $\pm$  SD, n = 3) of adult cuttlefish 1, 18, and 29 d after a single feeding on radiolabelled mussels.

Body compartments	1 d		18 d		29 d	
	<sup>241</sup> Am	<sup>134</sup> Cs	<sup>241</sup> Am	<sup>134</sup> Cs	<sup>241</sup> Am	<sup>134</sup> Cs
Branchial heart appendages	< 1	6 $\pm$ 9	< 1	1 $\pm$ 0	< 1	2 $\pm$ 0
Branchial hearts	3 $\pm$ 0	1 $\pm$ 0	< 1	2 $\pm$ 1	< 1	1 $\pm$ 1
Gills	1 $\pm$ 1	3 $\pm$ 2	< 1	2 $\pm$ 1	< 1	2 $\pm$ 1
Digestive tract	1 $\pm$ 1	3 $\pm$ 1	< 1	6 $\pm$ 0	< 1	9 $\pm$ 1
Genital tract	< 1	2 $\pm$ 1	< 1	9 $\pm$ 1	< 1	10 $\pm$ 6
Ovary	< 1	1 $\pm$ 1	< 1	3 $\pm$ 1	< 1	5 $\pm$ 2
Ink sack	< 1	1 $\pm$ 0	< 1	1 $\pm$ 0	< 1	2 $\pm$ 1
Skin	< 1	1 $\pm$ 0	< 1	2 $\pm$ 1	< 1	2 $\pm$ 0
Digestive gland	89 $\pm$ 7	31 $\pm$ 6	97 $\pm$ 1	57 $\pm$ 8	98 $\pm$ 0	54 $\pm$ 12
Kidney	< 1	1 $\pm$ 0	< 1	4 $\pm$ 1	< 1	2 $\pm$ 0
Muscle	6 $\pm$ 8	22 $\pm$ 3	1 $\pm$ 0	6 $\pm$ 5	< 1	5 $\pm$ 2
Head	2 $\pm$ 1	28 $\pm$ 6	1 $\pm$ 0	6 $\pm$ 5	1 $\pm$ 0	6 $\pm$ 2
Cuttlebone	< 1	1 $\pm$ 0	< 1	2 $\pm$ 1	< 1	2 $\pm$ 0

*Juveniles exposed via sea water*



*Juveniles fed brine shrimp*



*Adults fed mussels*

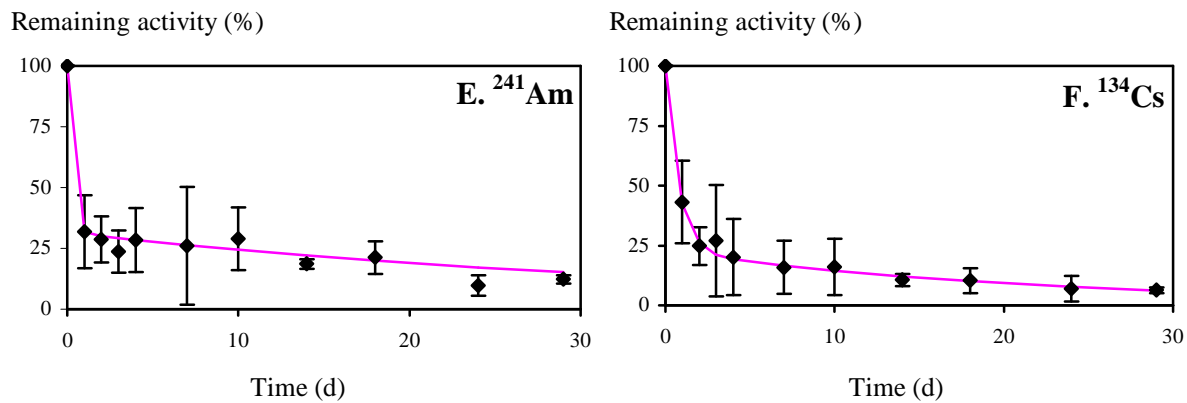


Fig. 1