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Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis* exposed to ^{203}Hg radiolabelled seawater and food

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ABSTRACT: Uptake and depuration kinetics of inorganic mercury (Hg) were investigated in the juvenile common cuttlefish *Sepia officinalis* following exposures via seawater and food using a sensitive radiotracer technique (^{203}Hg). Cuttlefish readily concentrated ^{203}Hg when exposed via seawater, with whole body concentration factors >260 after only 10 d of exposure. The total Hg accumulated from seawater was depurated relatively fast with a radiotracer biological half-life ($Tb_{½}$) of 17 d. During both exposure and depuration periods, accumulated Hg was mainly (>70%) associated with the muscular parts of the cuttlefish. However, the proportion of the whole-body Hg content associated with the digestive gland increased during exposure and depuration phases, suggesting that the metal was transferred from the muscles towards this organ for detoxification. When fed with radiolabelled food, cuttlefish displayed high assimilation efficiency (>90%) and the metal was found to be mainly located in the digestive gland (60% of the whole Hg content). Nevertheless, high depuration rates resulted in short $Tb_{½}$ (i.e. 4 d), suggesting that this organ has a major role in Hg detoxification and depuration. Whatever the exposure pathway, a low proportion of Hg (<2%) was found in the cuttlebone. Assessment of the relative contribution of the dietary and dissolved exposure pathways to inorganic Hg bioaccumulation in juvenile cuttlefish revealed that Hg was mainly accumulated from food, which contributed 77 ± 16% of the global metal bioaccumulation.

KEY WORDS: Mercury · Bioaccumulation · Kinetics · Body distribution · Cephalopod · Relative contribution

INTRODUCTION

Mercury (Hg) is one of the metals of highest concern in the marine environment as it is readily methylated by microorganisms, bioaccumulates in marine biota and consistently biomagnifies along the food chain (Cossa et al. 1990). Among marine organisms, most of the available information on Hg is related to fish, mainly because of their importance as a food source for humans. In fish, most of the Hg (>95%) is methylated and is therefore bioavailable to the upper trophic levels (Bloom 1992). Hence, fish consumption is an important source of Hg for humans (Svensson et al. 1992) and is a particular health concern (Clarkson 1990).

In contrast to fish, information on Hg in cephalopod tissues is scarce, despite the fact that these molluscs represent an increasing component of the world's fisheries (Boyle & Rodhouse 2006, FAO 2007). In addition, information on Hg in cephalopods is essentially limited to the main species targeted by fisheries and studies mainly report metal levels in edible tissues, i.e. mantle muscle, arms and fins (e.g. Buzina et al. 1989, Sapunar et al. 1989, Plessi et al. 2001). Recently, a study on a large range of cephalopod species from North Eastern Atlantic waters reported that Hg concentrations varied by 2 orders of magnitude among cephalopod species and that the metal was mainly stored in organic form in the muscular tissues (Bustamante et al. 2006). Several

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other studies on cephalopods from the Mediterranean suggest that these molluscs are able to accumulate high Hg concentrations in their tissues (Renzoni et al. 1973, Rossi et al. 1993, Storelli & Marcotrigiano 1999). Various factors are likely to influence Hg concentrations in cephalopods among which the animal size seems to be of primary importance (Monteiro et al. 1992, Rossi et al. 1993, Pierce et al. 2008).

Although it has been suggested that food is the main source of Hg accumulation in cephalopod tissues (Bustamante et al. 2006), the relative contribution of dietary and waterborne pathways has not been assessed in cephalopods. Moreover, as cephalopods are short-lived species, they might be interesting as short-term indicator species for the variation in environmental Hg concentrations (Seixas et al. 2005, Pierce et al. 2008).

For these reasons, the aim of the present study was to investigate the biokinetics of Hg uptake and depuration in cephalopods in order to better characterize its bioaccumulation, tissue distribution and retention capacity. The common cuttlefish *Sepia officinalis* was selected as a model species to study Hg transfer in cephalopods from seawater and food.

MATERIALS AND METHODS

Study organisms and radiotracer. Adult cuttlefish were collected by net fishing off Monaco in March and April 2006. They were acclimated and maintained in open-circuit tanks at the International Atomic Energy Agency, Marine Environment Laboratories (IAEA-MEL). After mating, the eggs laid by a single female were separated to optimise their oxygenation and kept in aquaria during the whole embryonic development (constantly aerated open circuit; flux: 50 l h^{-1}; salinity: 37 psu; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1; 12 h light:12 h dark cycle). Hatching occurred approximately 50 d after spawning. Young cuttlefish were then maintained in the same aquarium and fed with brine shrimp *Artemia* sp. for 5 d before the experiments.

The radiotracer ^{203}Hg (as $^{203}HgNO_3$; half life, $T_{½}$ = 46.59 d) was purchased from Isotope Products Laboratories. Stock solutions were prepared in 1 N nitric acid to obtain final radioactivity allowing the use of spikes of only a few microliters (typically 5 µl).

Experimental procedure. ***Contamination via seawater:*** Juveniles (n = 23; mean weight ± SD = 0.258 ± 0.009 g) were placed for 10 d in a 20 l glass aquarium containing 0.45 µm filtered natural seawater (constantly aerated closed circuit; temperature: 17°C; salinity: 37 psu; 12 h light:12 h dark cycle) spiked with ^{203}Hg (0.6 kBq l^{-1}). In terms of stable metal, this concentration corresponded to 20 ng l^{-1}. To facilitate the recurrent counting of each individual during the experiment, the juveniles were held individually in separate circular plastic containers (10 × 5 cm, diameter × height) covered with a mesh plastic net to allow for free water circulation. Radiotracer and seawater were renewed every second day to maintain water quality and keep radiotracer activity constant. Radiotracer activities in seawater were checked before and after each water renewal in order to determine the time-integrated radiotracer activities (Warnau et al. 1996, Rodriguez y Baena et al. 2006a). Juveniles were separated in 2 groups: the first group contained 16 tag-identified individuals and the second was composed of unidentified animals for the body distribution analyses. At different time intervals, radiotracer activities were counted in the same tag-identified juveniles (n = 16) throughout the experiment. According to their physiological state, individuals were removed from the experiment according to the following sampling plan: n = 16 from Days 0 to 3 and n = 14, 7 and 6 at Days 6, 9 and 10, respectively. In addition, after 3 and 9 d of exposure, 3 juveniles of the second group were counted and dissected to determine the radiotracer distribution among the digestive gland, the cuttlebone and the remaining tissues.

After this exposure period, the 6 remaining identified and radiolabelled juveniles (n = 6 from Days 0 to 4, n = 4 at Day 6 and n = 1 at Day 11) were held for 11 d in clean, flowing water (open circuit with constant aeration; seawater flux: 50 l h^{-1}; temperature: 17°C; salinity: 37 psu; 12 h light:12 h dark cycle). At different time intervals during the depuration period, the same identified juveniles were counted to establish the depuration kinetics of the radiotracer.

Contamination via food: Brine shrimp *Artemia* sp. were exposed for 5 d in a plastic aquarium containing 4 l of natural seawater spiked with ^{203}Hg until pools of 25 brine shrimp reached 3.1 kBq g^{-1} wet weight. The organisms were subsequently used as food for the juvenile cuttlefish.

As detailed in the seawater experimental procedure, juveniles were separated into 2 groups of identified and non-identified animals which were devoted to the depuration kinetics and body distribution studies, respectively. Hence, 16 newly hatched cuttlefish (mean weight ± SD = 0.297 ± 0.011 g) were placed in individual plastic containers (10 × 5 cm, diameter × height), and held in a 20 l aquarium under the same conditions as in the previous experiment. Each juvenile was fed for 1 h with 25 of the previously radiolabelled *Artemia* sp. At the end of the feeding period, the cuttlefish were immediately counted. From that time on, the cuttlefish were fed twice a day with uncontaminated *Artemia* sp. for 1 mo and regularly counted to determine radiotracer depuration kinetics and assimilation efficiency.

As mentioned above, juveniles showing poor health condition were removed leading to a sample number decrease throughout the experiment: n = 16 from Days 0 to 2, n = 14 from Days 3 to 14, n = 4 from Days 17 to 22 and n = 1 from Day 24 on. Throughout the depuration period, faeces were removed twice a day to reduce possible radiotracer recycling through leaching from the faeces. In addition, after 3 h, 9 d and 22 d of exposure, 3 other juveniles were counted and dissected to determine the radiotracer distribution among the digestive gland, the cuttlebone and the remaining tissues.

Radioanalyses and data treatment. Radioactivities were measured using a high-resolution γ-spectrometry system consisting of 4 coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with spectra analysis software (Interwinner® 6). The detectors were calibrated with an appropriate standard for each counting geometry used and measurements were corrected for background and physical decay of the radiotracer. Counting times were adapted to obtain relative propagated errors less than 5% (Rodriguez y Baena et al. 2006b). They ranged from 10 to 30 min for whole juveniles and from 10 min to 24 h for the dissected tissues.

Uptake of ^{203}Hg from seawater was expressed as change in concentration factors (CF; ratio between radiotracer content in the juvenile, Bq g^{-1}, and time-integrated activity in seawater, Bq g^{-1}) over time (Warnau et al. 1996). Uptake kinetic was best described by a saturation equation:

$$CF_t = CF_{ss}(1 - e^{-k_e t}) \quad (1)$$

where CF_t and CF_{ss} are the concentration factors at time t (d) and at steady state ($_{ss}$), respectively, and k_e is the biological depuration rate constant (d^{-1}) (Whicker & Schultz 1982).

Radiotracer depuration kinetics were expressed in terms of change in percentage of the remaining activity (i.e. radioactivity at time t divided by initial radioactivity measured in the organisms or in the tissue at the beginning of the depuration period × 100) over time. The depuration kinetic was best fitted by a mono-exponential equation:

$$A_t = A_0 e^{-k_e t} \quad (2)$$

where A_t and A_0 are the remaining activities (%) at times t (d) and 0, respectively. The determination of k_e allows the calculation of the radiotracer biological half-life ($Tb_{½} = \ln 2/k_e$). In the context of the seawater and feeding experiments, A_0 represents the absorption ($A_{0,w}$) and the assimilation efficiencies (AE), respectively.

Bioaccumulation model. The relative contribution of each uptake pathway was determined using the bioaccumulation model originally proposed by Thomann (1981) and revised by Thomann et al. (1995) and Metian et al. (2008). In this model, the total concentration of radiotracers in the juveniles, C_t (Bq g^{-1}) is equal to the sum of each concentration resulting from the uptake by the different pathways (Eq. 3):

$$C_t = C_{f,ss} + C_{w,ss} \quad (3)$$

where $C_{f,ss}$ is the food-derived radiotracer concentration (Bq g^{-1}) in juveniles at steady state (Eq. 4) and $C_{w,ss}$ the water-derived radiotracer concentration (Bq g^{-1}) in juveniles at steady state (Eq. 5):

$$C_{f,ss} = (AE \times IR \times C_f)/k_{e,f} \quad (4)$$

$$C_{w,ss} = (A_{0,w} \times k_{u,w} \times C_w)/k_{e,w} \quad (5)$$

where $A_{0,w}$ is the absorption efficiency (%) of the radiotracer from seawater, AE is the assimilation efficiency (%) of the radiotracer from food, C_f and C_w are the radiotracer activities in food and seawater (Bq g^{-1} and Bq ml^{-1}, respectively), respectively, IR is the ingestion rate (g g^{-1} d^{-1}), $k_{u,w}$ is the uptake rate constant (d^{-1}) from seawater and $k_{e,f}$ and $k_{e,w}$ are the biological depuration rate constants (d^{-1}) for food and water pathways, respectively.

The relative contribution (%) of each uptake pathway is then assessed from the following relationships:

$$\% \text{ food} = C_{f,ss}/(C_{f,ss} + C_{w,ss}) \quad (6)$$

$$\% \text{ seawater} = C_{w,ss}/(C_{f,ss} + C_{w,ss}) \quad (7)$$

Constants (and their statistics) of the best fitting equations (decision based on ANOVA tables for 2 fitted model objects) were estimated by iterative adjustment of the models using the nls curve-fitting routine in R freeware. The level of significance for statistical analysis was set at $\alpha = 0.05$.

RESULTS

Contamination through seawater

Uptake activity of ^{203}Hg in whole-body *Sepia officinalis* was best fitted by a saturation exponential equation with a calculated CF_{ss} of 480 (Fig. 1, Table 1). The CF actually measured (mean ± SD) at the end of the uptake period (CF_{10d}) of ^{203}Hg was 260 ± 70 (Table 2). Calculated CF_{10d} for the different organs indicated that ^{203}Hg was concentrated according to the following decreasing order: digestive gland (1460 ± 480) > remaining tissues (290 ± 80) > cuttlebone (47 ± 9). In terms of body distribution, ^{203}Hg was mainly found in the remaining tissues throughout the exposure period; this compartment accounted for 89 ± 3 and 80 ± 4% of the whole-body load of ^{203}Hg after 3 and 10 d of expo-

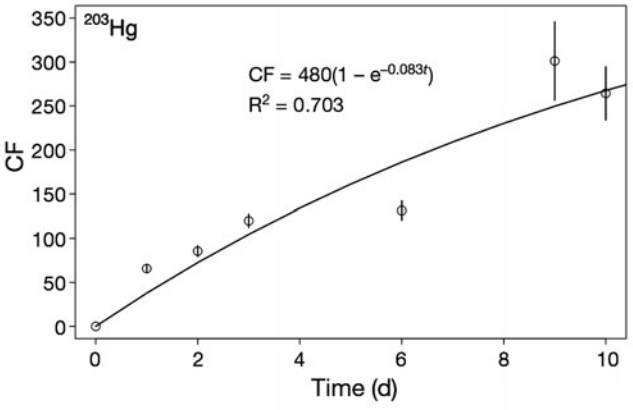

Fig. 1. *Sepia officinalis*. Whole-body uptake kinetics of ^{203}Hg in juvenile cuttlefish exposed for 10 d to the radiotracer dissolved in seawater. CF: concentration factor; n = 16 from Days 0 to 3 and n = 14, 7 and 6 at Days 6, 9 and 10, respectively

sure, respectively (Table 2). The cuttlebone presented very low Hg activity (<15 Bq g^{-1}) and loads (<3%), whereas Hg activity in the digestive gland increased with time, varying from 24 ± 4 Bq g^{-1} at Day 3 to 520 ± 170 Bq g^{-1} at Day 9, corresponding to 8 and 20% of the total radioactivity loads, respectively.

After the 10 d exposure period, non-contaminating conditions were restored and the depuration kinetic of ^{203}Hg was followed for 11 d. The whole-body depuration kinetic of ^{203}Hg in *Sepia officinalis* was best described by a mono-exponential model (Fig. 2, Table 1). This result indicated that 95% of the ^{203}Hg previously accumulated was depurated with a relatively short biological half-life (i.e. 17 d, Table 1). After 11 d of depuration conditions, most of the ^{203}Hg body load was associated with the remaining tissues (72%) while the digestive gland proportion increased up to 28% (Table 2).

Contamination through food

The depuration kinetic of ^{203}Hg ingested with food in *Sepia officinalis* was best fitted by a mono-exponential model, characterized by a biological half-life of 4 d (Fig. 2, Table 1) and allowed an estimated AE of 91%.

During the depuration period, the digestive gland displayed the highest proportion of the total body burden of ^{203}Hg, i.e. 68, 60 and 64% after 3 h, 9 d and 22 d of depuration, respectively (Table 2). The distribution of ^{203}Hg remained unchanged throughout the loss experiment, with the cuttlebone always showing the lowest proportion of the radiotracer (Table 2).

Bioaccumulation model

In order to assess the relative contribution of each uptake pathway to the global Hg accumulation in *Sepia officinalis*, the different kinetic parameters obtained for seawater and food experiments were used to feed the bioaccumulation model, along with other parame-

Table 1. *Sepia officinalis*. Whole-body uptake and loss kinetic parameters of ^{203}Hg in whole cuttlefish following different exposure experiments: (1) individuals (n = 16) were exposed for 10 d to the radiotracer in seawater then (2) placed in depuration conditions for 11 d (n = 6); (3) individuals fed on radiolabelled brine shrimp *Artemia* sp. were placed in depuration conditions for 30 d (n = 16). Uptake parameters — CF_{ss}: concentration factor at steady state; k_u: uptake rate constant (d^{-1}). All loss kinetics followed a mono-exponential depuration fit. Loss parameters — A_0: activity (%) lost according to the exponential component; k_e: depuration rate constant (d^{-1}); $Tb_{½}$: biological half-life (d, ±SE); p: probability of the model adjustment; ***p < 0.001

Condition	Uptake			Loss			
	CF_{ss}	k_e	R^2	A_0 (SE)	k_e	$Tb_{½}$	R^2
(1) Uptake seawater	480 ± 150	0.083 ± 0.036	0.703	–	–	–	–
(2) Loss seawater	–	–	–	95.3 (2.8) ***	0.041 ***	16.9 ± 3.9	0.472
(3) Loss food	–	–	–	90.7 (2.6) ***	0.180 ***	3.9 ± 0.3	0.804

Table 2. *Sepia officinalis*. Concentration factors (CF, mean ± SD) and tissue distribution (Dist., %, mean ± SD) of ^{203}Hg during seawater and feeding experiments

Tissue	Seawater contamination					Food contamination		
	Uptake 3 d		Uptake 10 d		Loss 11 d	Loss 3 h	Loss 9 d	Loss 22 d
	CF	Dist. (%)	CF	Dist. (%)	Dist. (%)	Dist. (%)	Dist. (%)	Dist. (%)
Digestive gland	110 ± 20	8.3 ± 2.6	1460 ± 480	19.7 ± 3.8	28.2	67.9 ± 10.6	59.9 ± 3.4	63.6 ± 5.8
Cuttlebone	29.7 ± 3.7	2.8 ± 1.0	47.0 ± 8.8	<1	<1	1.4 ± 0.9	2.4 ± 1.9	9.9 ± 3.2
Remaining tissues	110 ± 10	88.9 ± 3.4	290 ± 80	79.5 ± 3.7	71.8	30.7 ± 9.9	37.7 ± 4.5	26.5 ± 3.3
Whole body	96 ± 15	100	260 ± 70	100	100	100	100	100
Sample size (n)	3		3		1	3	3	3

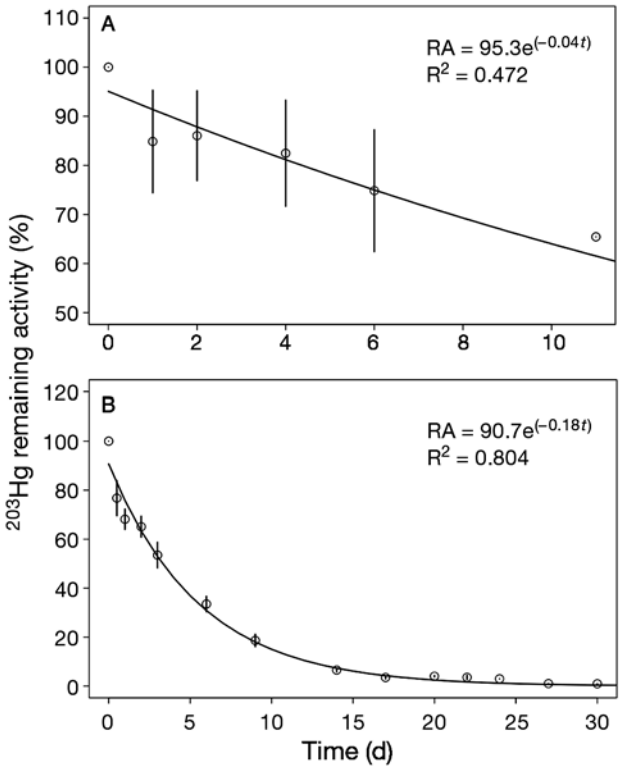

Fig. 2. *Sepia officinalis*. Whole-body loss kinetics of ^{203}Hg (% remaining activity [RA]; mean ± SD) in juvenile cuttlefish (A) previously exposed to radiolabelled seawater for 10 d (n = 6 from Days 0 to 4, n = 4 at Day 6 and n= 1 at Day 11) and (B) previously fed with radiolabelled brine shrimp (n = 16 from Days 0 to 2, n = 14 from Days 3 to 14, n = 4 from Days 17 to 22 and n = 1 from Day 24 on). Parameters for the best fitting equations are given in Table 1

ters such as the ^{203}Hg concentration in seawater and food (C_w = 0.364 Bq ml^{-1} and C_f = 3100 Bq g^{-1}, respectively) and the ingestion rate (IR = 0.07 g g^{-1} d^{-1}, congruent with the IR value determined by Koueta & Boucaud-Camou 1999). Modelling showed that food represented the main pathway for ^{203}Hg bioaccumulation in juvenile cuttlefish, contributing 77 ± 16% of the global metal bioaccumulation vs. 23 ± 14% for the seawater pathway.

DISCUSSION

Cephalopods are an increasing marine resource for world fisheries (Boyle & Rodhouse 2006). In the 1990s alone there was a 40% increase in squid catches worldwide (FAO 2007). Cephalopods are well-known for their capacity to accumulate high levels of non-essential metals, especially Ag and Cd, in their tissues (e.g. Martin & Flegal 1975, Bustamante et al. 1998, 2008). Hence the intake of contaminants such as metals by humans through cephalopod consumption is a matter of concern (e.g. Pierce et al. 2008). Some studies have also reported high concentrations of Hg in cephalopods from areas naturally contaminated by cinnabar, such as the Tyrrhenian and Adriatic Seas (e.g. Renzoni et al. 1973, Rossi et al. 1993, Storelli & Marcotrigiano 1999). However, the dynamic of Hg incorporation and its metabolism in cephalopods remain poorly understood. In the field, Hg could be methylated, which generally increases its toxicity as a result of its enhanced capacity for penetration across cell membranes (Boudou et al. 1983). The known methylated Hg uptake pathways in the biota are the transfer from the sediment, where sediment-associated bacterial flora are able to methylate part of the inorganic Hg (Compeau & Bartha 1985), and the consumption of prey such as juvenile shrimp, crab or fish, where Hg is stored under the methylated form in variable proportions. Considering that sand burying is a transient cryptic behaviour of cuttlefish (Poirier et al. 2004) and the grain size of the sediment selected by these species did not favour the methylation of Hg, the inorganic form of the metal could also be a significant source of accumulation for this nectobenthic cephalopod. In the present study, uptake and depuration biokinetics of Hg were determined using inorganic carrier-free ^{203}Hg in order to measure metal fluxes in real time at environmentally realistic contaminant concentrations (Warnau et al. 1996).

As a typical cephalopod, the common cuttlefish *Sepia officinalis* has a high food intake requirement to sustain its elevated growth rate. Being active predators, cephalopods have a high digestion efficiency (Boucher-Rodoni et al. 1987) and food has been shown to constitute an important source of uptake for various trace elements such as Am, Cd, Co and Zn (e.g. Guary & Fowler 1982, Koyama et al. 2000, Bustamante et al. 2002a, 2004). However, seawater could be an important bioaccumulation pathway as well, as elements can be taken up efficiently through the skin and the gills. For instance, seawater is the main intake pathway for Ag in adult *S. officinalis* (Bustamante et al. 2004). Therefore, there was a need to provide insights on the bioaccumulation of Hg under controlled experimental conditions in order to delineate the contribution of its uptake via the dissolved and dietary pathways.

After 10 d of exposure to dissolved ^{203}Hg, cuttlefish displayed quite elevated whole-body radiotracer activities (CF = 260 ± 70), indicating that Hg was efficiently bioconcentrated from seawater (Table 2). The estimated steady-state equilibrium (CF_{ss} = 480 ± 150) was reached after 2 wk of exposure. Among the 3 considered compartments, the digestive gland displayed the highest concentration capacity of ^{203}Hg (CF = 1460 ± 480; Table 2) whereas the cuttlebone showed the low-

est CF (i.e. <50; Table 2). This pattern is very similar to experimental data reported for other metals such as Ag, Cd, Co and Zn (Bustamante et al. 2002a, 2004). However, in terms of body burden distribution, ^{203}Hg was mainly stored in the remaining tissues (up to 80% of the total Hg load; Table 2), which are mainly composed of muscles, although they include the skin and the respiratory organs (i.e. gills), tissues directly exposed to the contaminated seawater. Likewise, Bustamante et al. (2006) reported that 70 to 90% of the total Hg body burden was stored in the muscular tissues in different cephalopod species collected from the North East Atlantic. This could be explained by the fact that (1) these tissues represents more than 70% of the total body weight and (2) Hg has a stronger affinity for the sulphydryl groups of muscular proteins rather than of fat tissue found in fish and/or cephalopod (Bloom 1992, Bustamante et al. 2006).

When *Sepia officinalis* was exposed to dissolved Hg, the digestive gland contained 8 and 20% of the total Hg burden after 3 and 10 d of exposure, respectively (Table 2). Therefore, the increasing proportion of Hg found in this organ implied that the digestive gland accumulates Hg more efficiently than the other compartments. Additionally, under depuration conditions, ^{203}Hg was released following a mono-exponential model and whole-body depuration was relatively rapid with a $Tb_{½}$ of approximately 17 d. After 11 d of depuration under running seawater, the tissues in direct contact with seawater contained significantly less ^{203}Hg than at the beginning of the depuration period—values varied from (mean ± SD) 135 ± 15 to 95 ± 17 Bq g^{-1} at Days 0 and 11 of the depuration period, respectively—whereas digestive gland activity remained unchanged (i.e. from 520 ± 170 Bq g^{-1} at Day 0 to 560 ± 30 Bq g^{-1} at Day 11; data not shown). These results suggest that (1) the digestive gland shows a stronger retention capacity for Hg than the other compartments and/or (2) a metal translocation occurred from the remaining tissues towards the digestive gland. In cephalopods, the digestive gland obviously plays a major role in the digestive processes, but it is also important in the detoxification of xenobiotics. Indeed, this organ has already been shown to retain translocated metals from other tissues (Bustamante et al. 2002a, 2004). The digestive gland is involved in the storage and detoxification of several metals such as Ag, Cd, Cu and Zn (e.g. Miramand & Bentley 1992, Bustamante et al. 2002b, Dorneles et al. 2007) and persistent organic pollutants such as PCBs (Ueno et al. 2003, Danis et al. 2005, Storelli et al. 2006). However, due to the fact that this organ does not store Hg in large amounts as shown for different cephalopod species in the field (Bustamante et al. 2006), the digestive gland might be also involved in the depuration of Hg when cuttlefish are exposed to the dissolved metal.

In the case of dietary exposure, the AE of Hg ingested with food was found to be nearly 100% (Table 1). This high degree of Hg assimilation might be due to the very efficient digestive metabolism that characterizes juvenile cuttlefish (Mangold 1989). Indeed, this early life stage is characterized by a predominant intracellular digestion process (compared to the extracellular digestion which is dominant in adults; Boucaud-Camou & Roper 1995), which could favour the metal assimilation. Such extreme assimilation efficiency has already been documented in cuttlefish: for instance, 90 and ~100% AE for Cd and Co, respectively, were reported in juveniles fed brine shrimp (Bustamante et al. 2002a, 2004). Nevertheless, the assimilated Hg was rapidly depurated with a $Tb_{½}$ of 4 d, which suggests that the processes governing Hg elimination are particularly efficient as well. Indeed, the ^{203}Hg activities in the digestive gland dropped from 310 ± 80 to 36 ± 6 Bq g^{-1} during the 22 d depuration period after feeding (data not shown). These results highlight the efficient excretion capacity of the digestive gland for Hg. Because, following dietary exposure, the main fraction of the whole Hg body burden was associated with this organ, it is not surprising that the retention time of Hg following dietary exposure was 4 times lower than following seawater exposure, i.e. 4 vs. 17 d (Table 1).

Under our experimental conditions, it appeared that the exposure of cuttlefish to contaminated seawater led to an accumulation of Hg in the remaining tissues of juveniles (>80%), mainly composed of muscular tissues. At the same time, Hg was translocated to the digestive gland and subsequently eliminated from the organism. Following dietary exposure, inorganic Hg was assimilated via the digestive gland and then rapidly eliminated. Consequently, the storage and/or redistribution of the bioaccumulated metal towards the remaining tissues are limited in cuttlefish. Nonetheless, considering both seawater and dietary exposure, food appears to be the predominant pathway for inorganic Hg bioaccumulation in juvenile cuttlefish. This result is not surprising considering the relatively high ingestion rate and the efficient digestive metabolism of cephalopods (Lee 1994).

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