Cadmium and mercury in cephalopod molluscs: Estimated weekly intake

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Abstract
Cadmium and mercury concentrations were measured in the flesh and hepatopancreas of different species of cephalopod molluscs (European squid, common octopus, curled octopus, horned octopus, pink cuttlefish, common cuttlefish) in order to establish whether the concentrations exceeded the maximum levels fixed by the European Commission. In hepatopancreas, the levels of cadmium were substantially higher than those in flesh (flesh 0.11–0.87 \( \mu \)g g\(^{-1}\) wet weight, hepatopancreas 2.16–9.39 \( \mu \)g g\(^{-1}\) wet weight), whilst the levels of mercury (flesh 0.13–0.55 \( \mu \)g g\(^{-1}\) wet weight, hepatopancreas 0.23–0.79 \( \mu \)g g\(^{-1}\) ) were approximately double those in flesh. Concentrations exceeding the maximum permitted limit of cadmium were found in 39.8 and 41.0% of common octopus and pink cuttlefish flesh, respectively. For mercury, concentrations above the limit were found only in octopuses, and precisely in 36.8, 50.0 and 20.0% of flesh samples of common, curled and horned octopus, respectively. In the hepatopancreas, concentrations of cadmium and mercury were above the proposed limits in all the samples examined. The estimated weekly intake of between 0.09 and 0.49 \( \mu \)g kg\(^{-1}\) body weight for cadmium and between 0.05 and 0.24 \( \mu \)g kg\(^{-1}\) body weight for mercury made only a small contribution to the provisional tolerable weekly intake (cadmium 1.3–7.0%, mercury 1.0–4.8%) set by the WHO.

Keywords: Cadmium, cephalopods, flesh, hepatopancreas, mercury

Introduction
In 1972, the World Health Organization of the United Nations issued a provisional tolerable weekly intake (PTWI) for cadmium and total mercury of 400–500 \( \mu \)g and of 300 \( \mu \)g per person, respectively (WHO 1972). The following toxicological re-evaluations maintained these PTWIs, although expressed in terms of intake per kg body weight (bw) (cadmium = 7 \( \mu \)g kg\(^{-1}\) bw; total mercury = 5 \( \mu \)g kg\(^{-1}\) bw) (WHO 1993, 2003). The need to establish preventive measures regarding the human intake of these metals originates from the fact that they are responsible of a wide variety of adverse effects. Occupational cadmium exposure is associated with lung cancer in human, and in some studies, occupational or environmental exposure to this metal has been associated with the development of cancer of prostate, kidney, liver, haematopoietic system and stomach (Ikeda et al. 2000; Waalkes 2000). In addition, the hazardous effects of mercury on human health have been clearly documented. This element is a neurotoxic agent that can cause serious nervous system damage, including psychological disturbance, impaired hearing, loss of sight, ataxia, loss of motor control and general debilitation (Aschner 2002). Food constitutes an obvious mean of human exposure to these metals. Recent surveys identified vegetables and cereals as the main contributors to the dietary intake of cadmium (Cuadrado et al. 2000; Watanabe et al. 2000), while seafood consumption appears to constitute a major route of mercury exposure for human, resulting in statistical differences between sub-populations with high and low fish consumption (Holsbeek et al. 1996; Nakagawa et al. 1997). Among seafood, mollusc cephalopods do not appear to play an important role as mercury vectors, whereas they might constitute a significant source
Materials and methods

In June–August 2004, 1412 specimens of mollusc cephalopods belonging to different species—*Loligo vulgaris* (European squid), *Octopus vulgaris* (common octopus), *Eledone cirrhosa* (curled octopus), *E. moschata* (horned octopus), *Sepia orbignyana* (pink cuttlefish) and *S. officinalis* (common cuttlefish)—were caught from the Adriatic Sea during several trawl surveys. Specimens of each species were grouped into pools according to their size (European Communities 2001), estimated as the maximum level fixed by the European Commission Decision (Official Journal of the European Communities 2001), as the weekly intake, and it was compared with the PTWI recommended by the Joint FAO/WHO Expert Committee on Food Additives (WHO 2003).

Results and discussion

Cadmium and mercury concentrations in the flesh and hepatopancreas of the different species of cephalopod molluscs are presented in Table I. In hepatopancreas (range 2.16–9.39 μg g⁻¹) the levels of cadmium were substantially higher than those encountered in flesh (range 0.11–0.87 μg g⁻¹) (*p* < 0.001). Following the criteria proposed by Miramand and Bentley (1992) (ratio <10 = poorly concentrated; 10 < ratio < 50 = moderately concentrated; ratio > 50 = highly concentrated), the hepatopancreas/flesh cadmium concentration ratio (10 < ratio < 30) indicated levels moderately higher in the digestive gland than in flesh. The capability of cephalopod hepatopancreas to concentrate high quantities of cadmium has already been reported (Miramand and Guary 1980; Miramand and Bentley 1992) even in organisms from unpolluted waters (Bustamante et al. 1998b). This particular tendency of the hepatopancreas to accumulate cadmium naturally points to the existence of the human body burden of cadmium. In fact, the ability of these organisms to accumulate high levels of cadmium is known, particularly in the hepatopancreas (Storelli and Marcostrigiano 1999; Raimundo et al. 2004), which contains up to 98% of the cadmium total body burden of the animal (Bustamante et al. 2002). This is of special interest for public health because cephalopod hepatopancreas is considered a delicacy by many people. For example, in Southern Italy, cephalopods are eaten whole; whilst in Spain, after maceration of the whole animal, they are used as squid sauce. In this scenario, the importance is clear of monitoring the cadmium and mercury burdens not only in the mantle and arms, parts usually eaten, but also in the hepatopancreas of these marine organisms in order to safeguard public health. With this objective the present study measured the concentrations of cadmium and total mercury in the flesh and in the hepatopancreas of different species of cephalopod molluscs and it was ascertained whether the cadmium and mercury concentrations were below the maximum level fixed by the European Commission Decision (Official Journal of the European Communities 2001), estimated as the weekly intake, and it was compared with the PTWI recommended by the Joint FAO/WHO Expert Committee on Food Additives (WHO 2003).

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of preferential binding sites for this element. Bustamante et al. (2002) suggested that the major part of cadmium is associated with lysosomes and cytosolic proteins, which play a function key in the storage and detoxication of the element. A comparison of the cadmium levels in the species analysed revealed the highest mean concentrations in pink cuttlefish (flesh 0.78 μg g⁻¹, hepatopancreas 9.39 μg g⁻¹) and common octopus (flesh 0.87 μg g⁻¹, hepatopancreas 7.86 μg g⁻¹), while European squid presented the lowest mean levels of this metal (flesh = 0.11 μg g⁻¹, hepatopancreas 2.16 μg g⁻¹). The other species showed intermediate concentrations between 0.24 and 0.27 μg g⁻¹ and between 3.38 and 6.97 μg g⁻¹ in flesh and hepatopancreas, respectively. The heterogeneity of cadmium concentrations among the different species is not an unexpected finding. It is, in fact, generally accepted that cadmium accumulation in these organisms, controlled by detoxification and elimination mechanisms, also depends significantly on their geographical origin and their ecology (Tanaka et al. 1983; Bustamante et al. 1998b; Storelli and Marcor trigiano 1999; Craig and Overnell 2003; Raimundo et al. 2004). For example, the squid Todarodes sagittatus from the Faroe Islands contained very high cadmium concentrations, while the cadmium levels in the same species from the Bay of Biscay were 17 times lower (Bustamante et al. 1998b). Likewise, variation in cadmium concentrations among different cephalopods might be diet-related: benthic cephalopods (octopuses and cuttlefish) feeding mainly on bottom invertebrates (McQuaid 1994) should be richer in cadmium than those neritic and pelagic ones, which prey mainly on fish and other cephalopods (Collins and Pierce 1996). Our findings support the above-mentioned proposition, having encountered the lowest concentrations in squid, and the highest in octopuses and cuttlefish. In addition, the results were in agreement with those reported by others. Storelli et al. (2005) in different species of cephalopods reported cadmium concentrations within which our values fell. For the hepatopancreas of octopuses (11.5 μg g⁻¹) and cuttlefish (5.34 μg g⁻¹), Miramand and Bentley (1992) reported concentrations matching well with our values. Likewise, the levels of cadmium found in the present study for the whole octopus Eledone cirrhosa were comparable with those recorded by Bustamante et al. (1998b) in organisms of the same species (9.06 μg g⁻¹).

Concerning mercury, the levels in hepatopancreas (range 0.23–0.79 μg g⁻¹) were approximately double those encountered in flesh (range 0.13–0.55 μg g⁻¹). These differences in concentrations highlight the difference in the physiological function of the two tissues, evidencing the important role of hepatopancreas in storage, redistribution, detoxification or transformation of contaminants. A comparative examination of mercury data showed that the studied species did not differ significantly in their concentration for a given tissue. In fact, comparing the concentrations found in the hepatopancreas, significant differences were detected only for European squid that exhibited mean levels two-to-three times lower (0.23 μg g⁻¹) than the other species (range 0.47–0.79 μg g⁻¹) (p < 0.01). For flesh a different situation was found: octopuses presented higher mean levels (0.43–0.55 μg g⁻¹) than squid (0.13 μg g⁻¹) and cuttlefish (0.16 μg g⁻¹). Moreover,

### Table I. Cadmium and mercury concentrations (μg g⁻¹ wet weight) in the flesh and hepatopancreas of the different species of cephalopod molluscs.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of pools</th>
<th>Cadmium</th>
<th>Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Squid:</td>
<td></td>
<td>Flesh</td>
<td>Hepatopancreas</td>
</tr>
<tr>
<td>Loligo vulgaris,</td>
<td>10</td>
<td>0.04–0.22</td>
<td>0.11±0.10</td>
</tr>
<tr>
<td>European squid</td>
<td></td>
<td>0.02–0.49</td>
<td>0.13±0.14</td>
</tr>
<tr>
<td>Octopus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octopus vulgaris,</td>
<td>10</td>
<td>0.06–1.73</td>
<td>0.87±0.53</td>
</tr>
<tr>
<td>common octopus</td>
<td></td>
<td>0.18–1.01</td>
<td>0.43±0.30</td>
</tr>
<tr>
<td>Eledone cirrhosa,</td>
<td>4</td>
<td>0.02–0.46</td>
<td>0.25±0.19</td>
</tr>
<tr>
<td>curled octopus</td>
<td></td>
<td>0.20–1.01</td>
<td>0.55±0.34</td>
</tr>
<tr>
<td>Eledone moschata,</td>
<td>4</td>
<td>0.05–0.69</td>
<td>0.24±0.21</td>
</tr>
<tr>
<td>horned octopus</td>
<td></td>
<td>0.14–1.73</td>
<td>0.49±0.15</td>
</tr>
<tr>
<td>Cuttlefish:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepia orbigniana,</td>
<td>5</td>
<td>0.28–1.27</td>
<td>0.78±0.49</td>
</tr>
<tr>
<td>pink cuttlefish</td>
<td></td>
<td>0.09–0.29</td>
<td>0.16±0.11</td>
</tr>
<tr>
<td>Sepia officinalis,</td>
<td>4</td>
<td>0.10–0.42</td>
<td>0.27±0.08</td>
</tr>
<tr>
<td>common cuttlefish</td>
<td></td>
<td>0.03–0.27</td>
<td>0.16±0.12</td>
</tr>
</tbody>
</table>
within each family the variability in mercury concentrations from species to species was minimal. It has been mentioned that the variations of cadmium tissue levels may be ascribed to diet. In addition, mercury concentration differences might be related to feeding habits of benthic and pelagic cephalopod species. Nevertheless, if dietary differences were the sole reason why mercury burden varied among various species, it would be expected to have comparable mercury levels either in cuttlefish or in octopuses because both benthic species. Our findings do not meet the aforementioned hypothesis suggesting that other factors can play a crucial role on the process of bioaccumulation of this metal by organisms. For example, fish size is recognized to be of importance in determining the rate of physiological processes influencing uptake, distribution and elimination of mercury as larger, older fish have generally higher concentrations than smaller, younger fish (Storelli et al. 1998; Joiris et al. 1999; Storelli and Marcotrigiano 2000). In the case of cephalopod molluscs, data from the literature on the correlation of mercury concentrations in tissues with weight are contrasting. Some research reported that mercury in flesh increases with cephalopod mass (Rossi et al. 1993; Barghigiani et al. 2000), while other studies revealed a dependency of mercury concentration on size (Bustamante et al. 1998a; Raimundo et al. 2004). On this basis, in our case no investigation was carried on metal–size relationships; it is likely to presume that the small cuttlefish size might have affected mercury accumulation in its tissues.

The studies on the mercury content in these molluscs are strikingly sparse. However, the values found for flesh and hepatopancreas of octopuses were similar to those found in organisms either from the Adriatic (Buzina et al. 1995; Storelli and Marcotrigiano 1999, 2004) or from other areas of the Mediterranean (El Hraiki et al. 1992), while Barghigiani et al. (2000) in horned octopus flesh from an area of the northern Tyrrhenian affected by mercury contamination found substantially higher concentrations (1.82 μg g⁻¹).

For food safety purposes, authorities around the world have set a standard for metal concentrations in food. European legislation (Official Journal of the European Communities 2001) specifies that the maximum concentrations of cadmium and mercury in cephalopod molluscs should be 1.00 and 0.5 μg g⁻¹ wet weight, respectively. Relative to cadmium, it must be highlighted that the value set by the European Commission refers only to eviscerated cephalopod molluscs. Using the values mentioned above as a guideline, concentrations exceeding the maximum permitted limit of cadmium were found in 39.8 and 41.0% of common octopus and pink cuttlefish flesh, respectively. For mercury, concentrations above the limit were observed solely in octopuses, and precisely in 36.8%, 50.0% and 20.0% of flesh samples of common, curled and horned octopus, respectively. On the contrary, concentrations of cadmium and mercury in the hepatopancreas were above the proposed limits in all the samples examined. However, exceeding the food standard maximum does not necessarily mean that the item is unfit for consumption, as the levels are conservatively set for regulatory purposes and assume ‘worst case’ scenarios (Turoczy et al. 2001). Hence, a more meaningful assessment of potential health hazards for consumers may be obtained by calculating the weekly intake of these metals deriving from the consumption of these type of seafood and comparing it with the PTWI set by the Joint Expert Committee on Food Additives (WHO 2003).

Table II showed the results of estimates of the dietary intake of cadmium and mercury when it is assumed that one 26-g portion per week (25 g flesh and 1 g hepatopancreas) is consumed by a 60-kg adult (Buzina et al. 1995; Turrini et al. 2001). The consumption of the whole animal resulted in an estimated weekly intake between 0.09 and 0.49 μg kg⁻¹ body weight for cadmium and between 0.05 and 0.24 μg kg⁻¹ body weight for mercury.

<table>
<thead>
<tr>
<th>Species</th>
<th>Estimated weekly intake of cadmium</th>
<th>Estimated weekly intake of mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flesh</td>
<td>Hepatopancreas</td>
</tr>
<tr>
<td>Squid Loligo vulgaris, European squid</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Octopus Octopus vulgaris, Common octopus</td>
<td>0.36</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Eledone cirrhosa, Curled octopus</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Eledone moschata, Horned octopus</td>
<td>0.10</td>
</tr>
<tr>
<td>Cuttlefish</td>
<td>Sepia orbignyana, Pink cuttlefish</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Sepia officinalis, Common cuttlefish</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*25 g flesh and 1 g hepatopancreas (Turrini et al. 2001).
These levels, though small in comparison with PTWIs (cadmium 1.3–7.0%, mercury 1.0–4.8%) must not be neglected. This observation originates from some considerations. First, cephalopod fisheries have been developed in the last few decades to exploit new stocks as an alternative to the decrease in the traditional finfish fisheries due to over fishing and to answer to a modification in the population’s feeding habits (Piatkowski et al. 2001). Both factors can lead to increases of cephalopod consumption, subsequently leading to an increase in the exposure levels for consumers. For example, in our case, a serving of 100 g cephalopods with their hepatopancreas (4 g) per week can give rise to a intake rather high (cadmium 0.32–1.93 μg kg⁻¹ body weight; mercury 0.24–0.95 μg kg⁻¹ body weight), especially in relation to cadmium. Such a seafood consumption level is rather common in certain populations of the Mediterranean region (Buzina et al. 1995) and among certain consumer groups, such as professional fishers and their families (Valentino et al. 1995). Moreover, it must be considered that the estimated dietary intake does not take into account exposure from food other than cephalopod molluscs.

In conclusion, the data do not suggest that cephalopod consumption should be discouraged. However, concentrations of cadmium of dietary concern are present in the hepatopancreas and it is therefore recommended that this tissue not be consumed on a regular basis. It is also recommended that regular control is carried out of cadmium levels in seafood and, where necessary, appropriate measures should be taken for the protection of public health.

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