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Trace element levels in foetus–mother pairs of short-beaked common dolphins (*Delphinus delphis*) stranded along the French coasts

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Abstract

Tissues of foetus–mother pairs of common dolphins (*Delphinus delphis*) stranded along the French coasts (Bay of Biscay and English Channel) were analysed for their Cd, Cu, Hg, Se and Zn contents. In the kidneys, foetal Cd levels were extremely low, and strong relationships between Cu and Zn suggested the involvement of metallothioneins since early foetal life. The results also indicated a limited maternal transfer of Hg during pregnancy since levels in the tissues of foetuses were below 1 $\mu g\ g^{-1}$ w.wt. However, hepatic Hg levels in foetuses increased with body length, and were also proportionate to maternal hepatic, renal and muscular Hg levels. Lastly, affinities between Hg and Se in tissues would participate in Hg neutralisation in both mothers – through tiemannite granules – and fetuses – through reduced glutathione – counteracting the toxic effects linked to the particularly high quantities of methyl–Hg to which marine mammals are naturally exposed.

Keywords: Marine mammals; Mercury; Selenium; Pregnancy; North-Eastern Atlantic

1. Introduction

During the last thirty years, a considerable body of data has been built up on pollutants levels in marine mammals (e.g. Wagemann and Muir, 1984; Law, 1996; Borrell and Reijnders, 1999). Indeed, due to their longevity and their elevated position in marine food webs, these organisms are generally considered as the integrators of the contamination of the environment (Reijnders, 1988). Some concerns have already been raised regarding adverse effects of pollutants, which include reproductive failures (e.g. Reijnders, 1980, 1986). Among generally focused contaminants, non essential heavy metals like cadmium (Cd) and mercury (Hg) are widely dispersed in the environment, since they are released from both natural and anthropogenic sources (Nriagu, 1996). Food is the major route of uptake of these elements for marine mammals, and consequently adult dolphins often display high metal levels in their tissues (Aguilar et al., 1999). Hence, the question of the importance of toxic metal transfer from mother to offspring is raised. Indeed, metals could be transferred from mothers to fetuses – *via* the placenta – and to suckling calves – *via* the milk – affecting them during their most sensitive periods of development. Generally, important metal exposure of human offspring to methyl–Hg can affect their normal neuronal development (WHO, 1990) whereas high dose of Cd during the gestational period can produce growth retardation in rat calves (Rohrer et al., 1979; Baranski et al., 1982). Such an altered growth is known to be induced by zinc (Zn) deficiency in foetuses, which may be caused by elevated maternal Cd impregnation (Brzoska and Moniuszko-Jakoniuk, 2001).

Numerous laboratory experiments and epidemiological studies have enabled to better understand the modalities of transfer of trace elements to offspring and their associated toxic

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effects in rodents and humans (see the reviews of Bell, 1984 and of Chang, 1984). However, determining the impact of toxic contaminants in wildlife marine mammal populations is more difficult, because 1) causality relationships between contaminant levels and potential toxic effects are not evident to show *in situ*, and 2) sampling is opportunistic. Sample accessibility easily explains why little data is available on trace element bioaccumulation in foetuses of cetaceans and is generally limited to few individuals (Itano and Kawai, 1981; Honda and Tatsukawa, 1983; Fujise et al., 1988; Law et al., 1992; Caurant et al., 1993; Yang et al., 2004).

Samples for trace element measurements in cetaceans generally originate from two basic sources, i.e. strandings and direct or incidental catches. Strandings may be divided into single and mass events. In the first case, the chemical levels may not reflect sometimes those of healthy populations since stranded individuals could suffer from diseases or parasitisms and additionally exhibit a poor nutritional status (e.g. Frank et al., 1992; Olsson et al., 1994; Bennett et al., 2001; Das et al., 2004; Dehn et al., 2005). Such biases are likely to be excluded when using animals from mass living stranding events or accidental by-catches.

This study focussed at mother–foetus pairs of short-beaked common dolphins (*Delphinus delphis*) from the North-Eastern Atlantic. We used two sources of samples, for which metal levels are likely to correctly reflect those of the healthy population, i.e. 1) a mass living stranding event composed of females and their calves, which got accidentally trapped during the ebb tide in a shallow bay of the English Channel (Pleubian, North coast of Brittany, February 2002) and 2) single stranding events of accidentally by-caught dolphins along the Atlantic French coastline (2002–2003). In these waters, common dolphins typically feed on fish and cephalopods (Pierce et al., 2004), which may expose them to a contamination risk by Hg (Bloom, 1992; Bustamante et al., 2006) and Cd (Bustamante et al., 1998, 2002). This cetacean species reach 20–30 years old, and its gestation period is about 11 months (Collet, 1981; Murphy, 2004). Such characteristics make the common dolphin a suitable species to clarify the importance of trace element gestational transfer in marine mammals. The investigated non essential elements were Cd and Hg, plus essential elements interacting in their detoxification processes. Firstly, we focussed on copper (Cu) and zinc (Zn) measurements because they are contained in metallothioneins (MTs), which play a key role in Cd detoxification (see the review of Das et al., 2000a). Secondly, selenium (Se) levels were examined because of its well-known co-precipitation with Hg, which lead to accumulation of non toxic granules of tiemannite (HgSe) in the liver (e.g. synthesis of Law, 1996). The present objectives were 1) to provide trace element levels in foetus–mother pairs of common dolphins, and 2) to determine which factors are likely to influence metal accumulation in foetuses.

2. Material and methods

This study is based on 17 foetus–mother pairs of common dolphins stranded along the French coasts. Most of the specimens (i.e. 10 pairs) originated from the mass living stranding event composed of 47 females and their calves (Pleubian, February 2002). The 7 other pairs were collected following single stranding events along the Atlantic French coastline during January months of 2002 and 2003. Post-mortems were carried out by veterinarians or by trained biologists of the Centre de Recherche sur les Mammifères Marins (CRMM) from the University of La Rochelle, and results were included in the European program BIOCET reports (Pierce et al., 2004). The nutritional condition of these animals, established from visual assessment of dorsal muscles and blubber thickness, was good in most cases ($n=11$) and medium in the other cases ($n=6$). No individuals could be classified as showing a poor nutritional condition. Concerning single stranding events, carcasses were fresh (intact pigmentation, cloudy eyes, tern coloration of organs during dissection), except in the case of one individual, which was slightly decomposed (skin peeling, moderate smell of decomposition). Moreover, some external observations (broken rostrum, amputated flukes) have permitted to classify all these individuals as accidentally by-caught.

Note that before necropsy, total body length was measured (rostrum to fluke notch) and values for foetuses ranged from 32 to 60 cm. In the North-Eastern Atlantic, common dolphins have a length of 90–100 cm at birth (Collet, 1981; Murphy, 2004). Thus, foetuses from this study would have been collected at early to mid-pregnancy periods.

Various tissues and organs were sampled during necropsies. Teeth of mothers were collected in order to determine age, following the recommendations of Perrin and Myrick (1980). One GLG (Growth Layer Group) is considered to represent one year in common dolphins (Gurevich et al., 1980) and ages were recorded to the nearest 0.5 GLG. In addition, the kidneys and the liver of foetuses and mothers were systematically sampled and stored in plastic bags at −20 °C for trace element analyses. Muscle and blood samples were collected from a subset of mothers and added to the sampling in order to investigate modalities of Hg transfer to foetuses. Blood was directly collected from pregnant females ($n=9$) that died following the mass living stranding event and samples were stored into sterile polypropylene tubes.

With the exception of blood, all fresh samples were freeze-dried and ground to powder. The mean ratios between dry weight (d.wt.) and wet weight (w.wt.) were 0.30 for the muscle of mothers, and 0.22 and 0.28 for the liver, and 0.18 and 0.23 for the kidneys in foetuses and mothers, respectively. Each sample was then treated in duplicate. For total Hg measurements, aliquots ranging from 0.5 to 3 mg of dried-material, as well as thawed whole-blood from mothers, were analysed directly in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). For the other trace element analyses, 2 aliquots of approximately 200 mg of each homogenised dry sample were digested with 3.5 mL of 65% HNO_3 at 60 °C for 3 days. The digested contents were then diluted to 10 mL in milli-Q quality water. Then Cd, Cu and Zn contents were assayed using a flame (Varian 250 Plus) Atomic Absorption Spectrometer (AAS) with deuterium background correction whereas Se and some low Cd contents were analysed with graphite furnace AAS (Hitachi Z-5000) with Zeeman background correction. Quality controls were made using standard reference materials from National Research Council of Canada (NRCC), i.e. dogfish liver (DOLT-2 and DOLT-3), dogfish muscle (DORM-2), and lobster hepatopancreas (TORT-2). These reference materials were treated and analysed under the same conditions as the samples. Results were in good agreement with the certified values (Table 1). In addition, the laboratory participates in intercalibration exercises organised by the International Atomic Energy Agency (cf. Coquery et al., 1999). During the last exercise, our laboratory was classified in group 1, indicating the good quality of results for all analysed elements (Azemard et al., 2006). Detection limits ($\mu g\ g^{-1}$ d.wt.) were 0.004 for Cd, 0.5 for Cu, 3 for Zn, 0.8 for Se, and 0.005 for Hg. All concentrations below the detection limit were replaced with "dummy values" that were half of the detection limit in order to allow further statistical comparisons (Gibbons and Coleman, 2001). Metal levels in dolphin tissues were expressed as $\mu g\ g^{-1}$ w.wt.

Spearman rank correlation coefficients were used 1) to determine intermetallic relationships in the kidneys and the liver of foetuses, mothers, and between the mother–foetus pairs, and 2) to establish correlations between trace elements and length of foetuses or age of mothers. Particular attention was paid to Hg, for which foetus–mother relationships were investigated using maternal concentrations in blood, muscle, kidneys, and liver.

3. Results and discussion

Table 2 gathers mean trace element levels in the tissues and blood of foetus and mother common dolphins stranded along the French coasts. With the exception of Cd, all trace elements were present at higher