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Toxicological investigations on four sperm whales stranded on the Belgian coast: inorganic contaminants

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Abstract

Nine heavy metals have been analysed in the liver, muscle and kidneys of sperm whales that stranded on the Belgian coast, November 18, 1994. The concentrations of most of the studied inorganic contaminants – except mercury and cadmium – were low. The mercury content of the tissues was high, but in the range of those found in sperm whales previously described in the literature, as shown by JOIRIS *et al.* (this volume), and most of the mercury was found under an inorganic form. We found a close correlation between the mercury and selenium contents of the livers, which strongly suggests that the pollutant was detoxified under the tiemannite form, and therefore was not potentially toxic for the animals. On the contrary, cadmium was found in high concentrations, which was expectable owing to the normal diet of the species (cephalopods), but twice those previously described in the literature for sperm whales. Moreover, the metal was not found, as it is generally the case, to be bound to metallothioneins (a protein well known for its protective effect against heavy metals toxicity) and therefore may have contributed to the debilitation of the animals.

Keywords: sperm whale, heavy metals, detoxication, selenium, cadmium, debilitation.

Résumé

Neuf métaux lourds ont été analysés dans le foie, le muscle et les reins de cachalots qui s'échouèrent à la côte blege le 18 novembre 1994. Les concentrations de la plupart des contaminants inorganiques étudiés étaient basses, à l'exception du cadmium et du mercure. Comme le montrent JOIRIS *et al.* (ce volume), la charge des tissus en mercure était élevée, mais dans les limites de celles décrites dans la littérature chez le cachalot, et la plus grande partie du mercure se trouvait sous forme inorganique. On a trouvé une corrélation étroite entre la charge en mercure et en sélénium dans les foies, ce qui suggère fort que le polluant était détoxifié sous la forme de tiemannite et qu'il n'était donc potentiellement pas toxique pour les animaux. Au contraire, on a trouvé des concentrations élevées de cadmium - ce qui était prévisible étant donné le régime alimentaire habituel de cette espèce (céphalopodes) - mais deux fois supérieures à celles décrites antérieurement dans la littérature chez le cachalot. En outre, ce métal n'est pas apparu lié à des métallothionéines (une protéine bien connue pour la protection qu'elle offre contre la toxicité des métaux lourds) et par conséquent il peut avoir contribué à la délibitation des animaux.

Mots-clés: cachalot, métaux lourds, détoxication, sélénium, cadmium, débilitation.

Marine mammals are important top predators in marine ecosystems. According to their position in the trophic networks, their long life span and their long biological half-time of elimination of pollutants, they accumulate high levels of chemicals, e.g. heavy metals, which have been related to the occurence of several abnormalities, debilitation, immuno-suppression, infectious diseases and impaired reproduction. All together may lead to a long term decline of the populations. The cause of debilitation and/or death is probably multifactorial and related to all the environmental hazards the animals are submitted to. SWART *et al.* (1994) have demonstrated that impaired immunological functions in harbour seal were associated with chronic exposure to environmental contaminants accumulated through the marine food chain. However, estimation of the actual toxicity of one heavy metal in whales, from its content in tissue, is not obvious. A wide range of natural concentrations can be encountered in a single species in relation with age, sex and season, and no experimental data are available on these marine mammals. On the strength of comparative studies, it is believed that they are able to detoxify part of the heavy metals contained in their tissues.

It is a matter of fact that heavy metals can be stored and detoxified by marine animals by binding to specific proteins, by a compartmentation within membrane-limited vesicles (*e.g.* lysosomes) or by precipitation in specific granules (see BOUQUEGNEAU *et al.* (1984) and BOUQUEGNEAU & JOIRIS (1988)). Marine mammals are able to bind metals such as zinc, cadmium, copper and inorganic mercury to metallothioneins which are cytosolic low-molecular weight proteins with high cysteine content (see e.g. TOHYAMA *et al.* (1986)). As homeotherms, however, marine mammals have to consume large amounts of food and, owing to the important assimilation efficiency of the methylmercury contained in it, they naturally contain high levels of the pollutant and have had to develop detoxification processes of this very toxic compound. For this reason, the accumulation of large amounts of mercuric selenide granules can be considered an adaptation to dietary mercury (Fig. 1).

KOEMAN *et al.* (1973) first reported a functional relationship between mercury and selenium in marine mammals. LATERT, MARTOJA & BERRY (1980) identified particles of pure tiemannite (mercuric selenide) stored in

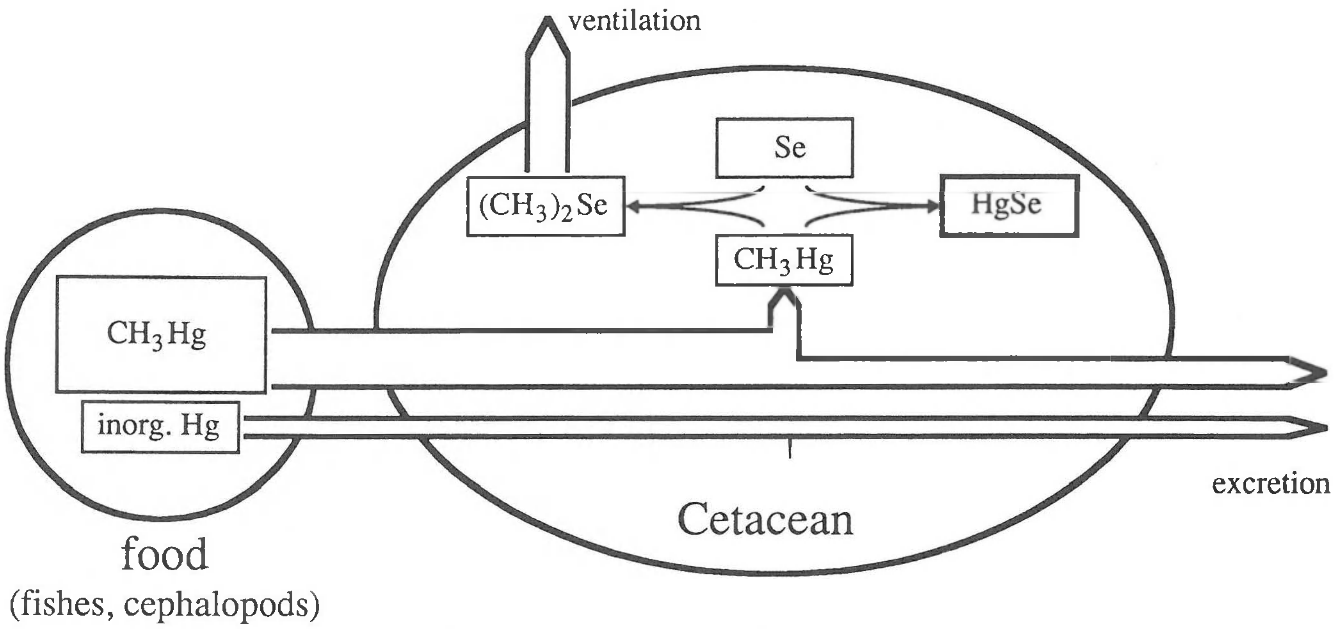

Fig. 1 – Fate of dietary mercury in cetaceans.

granules of the connective tissue of the liver of *Ziphius Cavirostris*. Since these works, many authors have confirmed both a correlation between selenium and mercury concentrations, and the presence of tiemannite in dense intracellular granules, not only in the liver, but also in the spleen, lungs, brain, muscle and kidneys of marine mammals (see *e.g.* NIGRO & LEONZIO (1993)).

These detoxifying mechanisms (binding to metallothioneins, compartmentation in membrane-limited vesicles, granule formation) lead to high but nontoxic concentrations of heavy metals in the tissues. The speciation of the metal during laboratory analysis is therefore needed to evaluate its potentially toxic concentration.

The liver, muscle and kidneys of the four sperm whales stranded on the Belgian coast, November 18, 1994, were analysed for nine heavy metals. The speciation of cadmium, copper, mercury and zinc was studied in respect with their binding to metallothioneins. The selenium content of liver and kidneys of the sperm whales has been analysed in order to assess to which extend mercury could be stored under the tiemannite form in the animals. Zinc, lead, nickel, cadmium, iron, chromium, copper and titanium were analysed by I.C.P.S., total mercury and selenium by flameless atomic absorption spectrophotometry and anodic stripping voltammetry respectively. Finally, in order to try to assess the debilitation of the animals, total lipids contents were also estimated. For the study of metal-binding to metallothioneins, the tissues were homogenized and then centrifuged at 26,000 g for 1 hour, to separate soluble and insoluble fractions. The supernatant was filtered on a LKB Ultrogel AcA 54 column. Copper, zinc , cadmium and mercury were analysed in the chromatographic fractions by atomic absorption spectrophotometry. Metallothioneins are indeed thermostable and resistant to proteolytic enzymes, and they can successfully be determined in the carcasses of dead animals (BOUQUEGNEAU *et al.*, 1996). Details of the results are reported in JOIRIS *et al.* (1995) and HOLSBEEK *et al.* (*in prep.*).

The debilitation of the stranded sperm whales, previously quoted, was first confirmed by the relatively low lipid content of the liver and muscles, compared with white-beaked dolphins and harbor porpoises stranded along the Belgian coast (JOIRIS *et al.*, 1995) and with sei whales in antarctic summer (BOTTINO, 1978).

Few data are available in the literature which would allow one to assess the potential toxicity of the metals contained in the sperm whales stranded at Koksijde on November 18th 1994. From our results and literature data about cetaceans (see THOMPSON, 1990) and sperm whales in particular (RIDLINGTON *et al.* (1981), NAGAKURA *et al.* (1974) and JOIRIS *et al.* (1991)), zinc, lead, nickel, chromium and copper concentrations are to be considered low, while mercury and cadmium levels appear to be high.

The mercury content of the muscle is important, but nevertheless in the range of both sperm whales from the North Pacific (NAGAKURA *et al.*, 1974) and the sperm whale which was found stranded in 1988 along the Belgian coast (JOIRIS *et al.*, 1991). Sperm whale No 1, probably the youngest individual among the four, displays a lower mercury concentration. The metal stored in the liver and kidneys is mainly under the inorganic form: less than 10 % was found under the methylated form in the liver, about 20 % in the kidneys (HOLSBEEK *et al.*, *in prep.*). No detectable amount of inorganic mercury was found to be bound to metallothioneins, but a close rela-

Table 1 – Mean cadmium concentration in the liver of cetaceans

Species	(mg/kgFW)	Main Diet
Harbour porpoise	0.2 [(1)]	fish
Beluga	0.9 [(1)]	fish
Bowhead whale	1.5 [(1)]	crustacea/pteropods
Bottlenose whale	5.6 [(1)]	cephalopods
Striped dolphin	6.3 [(1)]	fish/cephalopods
Sperm whale	12.0 [(2)]	cephalopods
Sperm whale	25.0 [(3)]	cephalopods
Narwhal	32.0 [(1)]	cephalopods
Ziphius	50.5 [(1)]	cephalopods
Pilot whale	69.4 [(1)]	cephalopods/fish

(1) compiled from Thompson (1990)
(2) from Ridlington *et al.* (1981)
(3) sperm whales stranded at Koksijde on November 18th 1994 (HOLSBEEK *et al.*, *in prep.*)

tionship was shown between selenium and mercury contents of the liver, which suggests a detoxification of methylmercury under the tiemannite form (HOLSBEEK *et al.*, *in prep.*).

On the contrary, the mean cadmium content in samples of the liver was high, twice that found in the North Pacific sperm whales described by RIDLINGTON *et al.* (1981), but however in the range of the liver cadmium concentration of mammal species that feed on cephalopods (Table 1).

This suggests a potential toxicity of cadmium, an inference that is strengthened by the study of the speciation of the metal: only a small part of the cadmium appears to be detoxified through binding to metallothioneins (10 % of total cadmium, whilst RIDLINGTON *et al.* (1981) found 92 % of cadmium bound to the metallothioneins of the livers of sperm whales stranded in the North Pacific). This suggests that cadmium, contrary to zinc and copper which are normally bound to metallothioneins, was potentially highly toxic for these animals.

To conclude, the four sperm whales stranded on the Belgian coast in November 1994 exhibited high levels of contamination by mercury and cadmium. Cadmium, which is known to induce debilitation in mammals and was not, as it is usually the case, detoxified by metallothioneins, is therefore to be considered as one of the factors responsible for the debilitation of these animals, a condition which could have favoured the stranding.

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