

## Heavy metals in marine mammals

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### 1. INTRODUCTION

During the past few decades, increasing concern about environmental pollution has led to many investigations on heavy metals and their distribution in the sea, air or biological materials. The distribution of xenobiotics in the marine environment is not homogeneous and a considerable variation of the concentrations may occur regionally and temporally. The use of bioindicators offers a useful alternative for pollution monitoring studies. Marine mammals appear to be potentially valuable indicators of the level of heavy metals accumulated in the marine environment: according to their top position in the trophic network, their long life span and their long biological half-time of elimination of pollutants, these animals accumulate high levels of chemicals, such as organochlorines (Tanabe et al., 1994; Kamrin and Ringer, 1994), or heavy metals (André et al., 1991a; Bouquegneau and Joiris, 1988; 1992; Dietz et al., 1998).

The increased interest in studying contaminants in marine mammals is due to large-scale die-off (Sarokin and Schulkin, 1992; Forcada et al., 1994) or impaired reproduction (De Guise et al., 1995), which could lead to population declines of some pinniped and small cetacean species in Europe and North America, and the finding of relatively large contaminant burdens in these animals. In many cases, morbillivirus infections were the primary cause of the disease outbreaks (Heide-Jørgensen et al., 1992; Thompson and Hall, 1993; de Swart et al., 1995a). These mass mortalities among seals and dolphins inhabiting contaminated marine areas have led to speculation about the possible involvement of immunosuppression associated with environmental pollution.

Controlled experiments are unavailable to establish any definite causal relation between these pollutant concentrations and any physiological problem. Moreover, the data are always difficult to interpret because of the presence of other chemical contaminants and other stressors. The physiological status of the organisms (e.g. pregnancy, molting, fasting...) also modulates the toxicity of heavy metals. In addition, the available measurements have almost been all performed on animals found dead, which leaves doubts about the general applicability of collected values at which an effect at individual or population level might be expected.

Several investigations have been carried out in an attempt to evaluate contaminant effects at ambient environmental levels (Reijnders, 1986; Aguilar and Borrel, 1994; De Guise et al., 1995; de Swart et al., 1995b; 1996). For example, it has been demonstrated that seals fed polluted fish from the Dutch Wadden Sea showed reduced pup production when compared to those fed much less polluted fish from the Northeast Atlantic (Reijnders et al., 1986). This study was the first sign of a causal relationship between naturally occurring levels of pollutants and a physiological response in marine mammals.

A more recent study over a two year period by de Swart et al. (1994; 1995b; 1996) has demonstrated an impairment of several immune parameters in harbour seals (*Phoca vitulina*) fed on herring from the polluted Baltic Sea when compared to those fed with fish from the Atlantic Ocean.

Among impaired parameters, natural killer cell activity plays an important role in the first line of defense against viral infections (de Swart et al., 1996). Moreover, those seals consuming contaminated herring accumulated higher body burdens of potentially immunotoxic organochlorines than seals fed relatively uncontaminated herring. In the latter study, heavy metal levels have unfortunately not been determined either in fish or in seals. Possible immunosuppressive actions of other groups of environmental contaminants, such as heavy metals, cannot be ruled out. Indeed, many laboratories and epidemiological studies have demonstrated the immunotoxic effects of heavy metals in a variety of species (Zelikoff and Thomas, 1998). Direct cause and effect links between a single kind of contaminant and possible population declines has not been established so far, so that many researchers have proposed the possibility of a synergistic role of different substances in increasing the susceptibility of affected animals to diseases or biotoxins.

In this chapter we will focus on heavy metals and their possible effects on marine mammals. Heavy metals are usually divided into essential (Zn, Cu, Cr, Se, Ni, Al) and non-essential metals (Hg, Cd, Pb), the latter being

**Table 5: Heavy metals in livers from other marine mammals: selected references**

Order	Family	Species	N	Location	Hg-total	CH <sub>3</sub> -Hg	Cd liver	Cd kidney	Se	Cu	Zn	Source
Sirenia	Dugongidae	<i>Dugong dugon</i>	6	Australia	/	/	/ <0.1-59	/ 0.2-309	/	/ 9-608	219-4183	Denton <i>et al.</i> , 1980
Carnivora	Ursidae	<i>Ursus maritimus</i>	8 (<7y.)	Northeast Greenland	86±5	/	7±6	79±6	36±5	/	/	Dietz <i>et al.</i> , 1996

All the data are expressed in dry weight. Because mean values were not available for dugong, only the range are displayed. The concentrations found in polar bears are expressed as mean ± standard deviation

**Table 4:** Heavy metals mean and range concentrations in livers from pinnipeds: selected references

Family	Species	Location	n	Hg-total	CH <sub>3</sub> -Hg	Cd liver	Cd kidney	Se	Cu	Zn	Source
Phocidae	<i>Pusa hispida</i>	Northwest Greenland	5	/	/	232 108-436	1596 1036-2324	/	/	/	Dietz <i>et al.</i> , 1998
		Swedish coasts	4	176 19-348	/	2.6 0.6-3.4	8 5-22	76 11-112	18 15-19	128 72-180	Frank <i>et al.</i> , 1992
	<i>Phoca vitulina</i>	Swedish coasts	8	104 5-264	/	0.4 0.2-0.7	2 1-3	44 16-104	34 6-52	216 76-248	Frank <i>et al.</i> , 1992
		Arctic	13	24 0.9-173	/	12 1-39	77 2-222	14 2-66	26 12-84	171 121-287	Wagemann, 1989
	<i>Halichoerus grypus</i>	Swedish coasts	9	104 92-368	/	0.7 0.3-0.8	5 2-11	56 39-208	56 28-72	184 140-304	Frank <i>et al.</i> , 1992
		Northwest England (Liverpool Bay)	12	590 6-1720	/	2 <0.06-4	/	/	53 9-112	218 88-356	Law <i>et al.</i> , 1992
	<i>Leptonychotes weddelli</i>	Antarctica	3	16 0.2-34	/	3 <0.02-5	17 <0.02-40	/	80 76-103	191 166-220	Yamamoto <i>et al.</i> , 1987
Otariidae	<i>Arctocephalus gazella</i>	Georgia	11	215 52-334	/	350 55-684	/	/	263 132-438	384 259-643	Malcom <i>et al.</i> , 1994
	<i>Arctocephalus philippii</i>	Chile	Pups : n=27 Adult : n=1	9 ± 6 75	/	0.5 ± 0.2 2	/ /	/ /	/ /	/ /	Sepulveda <i>et al.</i> , 1997
Odobenidae	<i>Odobenus rosmarus</i>	Arctic	114	4 0.03-19	/	38 0.1-137	244 0.1-564	10 2-20	32 6-137	151 50-300	Wagemann and Stewart, 1994

Cadmium concentrations are also given for the kidney. When the range was not available, standard deviation was used. All the data are expressed in dry weight, assuming a mean water content of 75% of the tissues.

**Table 3:** Heavy metal mean and range concentrations in livers from mysticetes: selected references

Family	Species	n	Location	Hg-total	CH <sub>3</sub> -Hg	Cd liver	Cd kidney	Se	Cu	Zn	Source
Eschrichtiidae	<i>Eschrichtius robustus</i>	10	Western North America	0.06 0.01-0.1	nd	4 0.06-6	4 0.1-6	2 0.3-3	9 0.6-25	99 2-160	Varanasi <i>et al.</i> , 1994
Balaenopteridae	<i>Balaenoptera acutorostrata</i>	135	Antarctic	0.2 0.1-0.5	nd	38 9-133	nd	nd	17 9-34	146 99-232	Honda <i>et al.</i> , 1986; 1987
		17	West Greenland	2 <sup>1</sup> 1-11	nd	4 <sup>1</sup> 2-6	15 <sup>1</sup> 7-22	6 <sup>1</sup> 4-10	nd	138 <sup>1</sup> 106-192	Hansen <i>et al.</i> , 1990
	<i>Balaenoptera physalus</i>	11	Northeast Atlantic	2 0.6-5	40%	nd	nd	nd	nd	nd	Sanpera <i>et al.</i> , 1993
Balaenidae	<i>Balaena mysticetus</i>	20	Alaska	0.2 0.08-0.4	nd	31 2-88	nd	4 1-9	20 12-40	137 88-261	Krone <i>et al.</i> , 1999

<sup>1</sup>: median

Cadmium concentrations are also given for the kidney. All the data are expressed in dry weight, assuming a mean water content of 75% of the tissues. nd: not determined

**Table 2:** Heavy metals mean and range concentrations in livers (and kidney for Cd) from odontocetes: selected references

Family	Species	Location	n (age estimation )	Hg-total	CH <sub>3</sub> -Hg	Cd liver	Cd kidney	Se	Cu	Zn	Source
Pontoporiidae	<i>Pontoporia blainvillei</i>	Argentina	2 (2and3y.)	15±6	nd	13±6	40±16	nd	64±9	330±160	Marcovecchio <i>et al.</i> , 1990
Platanistidae	<i>Platanista gangetica</i>	India	4	nd	nd	0.1 <0.04-0.15	2 <0.04-6	nd	207 9-400	126 64-210	Kannan <i>et al.</i> , 1993
Delphinidae	<i>Tursiops truncatus</i>	Argentina	1 (10y.)	344±29	nd	3±1	114±17	nd	310±1	785±136	Marcovecchio <i>et al.</i> , 1990
		South Carolina, USA	34	71 <2-586	nd	0.2 0.02-1	nd	38 0.7-189	43 5-316	227 46-1084	Beck <i>et al.</i> , 1997
		Gulf of Mexico	10 Adult males	180 20-351	nd	2 0.4-5	nd	74 41-133	nd	nd	Kuehl and Haebler, 1995
	<i>Globicephala melas</i>	Alaska	11	180 4-448	nd	nd	nd	52 6-114	nd	nd	
		Faroë Islands	Hg: n=8 Cd: n= 28	852±776	17±15%	308 6-668	344 6-976	nd 0-480	nd 8-60	nd 100-900	Caurant <i>et al.</i> , 1996 Caurant <i>et al.</i> , 1995
		Newfoundland, Canada	26	63 0.3-298	nd	78 0-190	43 0-102	31 3-113	17 9-35	264 68-716	Muir <i>et al.</i> , 1988
	<i>Stenella coeruleoalba</i>	Northeast Atlantic	Hg: n=8 Others: n=22	206 5-348	nd	17 0.2-51	91 0.1-199	nd	43 7-272	167 33-385	Das <i>et al.</i> , 2000 André <i>et al.</i> , 1991 <sup>b</sup>
		Pacific Ocean, Japan	mature dolphins n=15	820±408	3.4%	nd	nd	194±115	nd	nd	Itano <i>et al.</i> , 1984
	<i>Delphinus delphis</i>	Northeast Atlantic	28 Stranding	128 3-631	7%	6 0-96	13 0.4-81	nd	12 3-32	143 65-293	Holsbeek <i>et al.</i> , 1998
	<i>Lagenorhynchus albirostris</i>	Newfoundland	26	3 0.5-6	nd	2 0.2-8	14 2-44	8 4-12	20 3-32	100 43-136	Muir <i>et al.</i> , 1988

Table 2 (continued).

Family	Species	Location	n	Hg-total	CH <sub>3</sub> -Hg	Cd liver	Cd kidney	Se	Cu	Zn	Source
Monodontidae	<i>Monodon monoceros</i>	Baffin Island	38	24±12	/	133±130	298±192	16±7	21±13	151±40	Wagemann <i>et al.</i> , 1983
		West Greenland	n>48	21 <sup>1</sup> <0.02-171	/	43 <sup>1</sup> <0.06-295	156 <sup>1</sup> <0.06-500	13 <sup>1</sup> <0.8-144	/	144 <sup>1</sup> 53-271	Hansen <i>et al.</i> , 1990
	<i>Delphinapterus leucas</i>	West Greenland	40	7 0.3-123	/	9 <0.06-34	41	15 2-111	/	114 87-181	Hansen <i>et al.</i> , 1990
		St. Lawrence, Canada	30	134 1.5-808	/	0.6 0.004-2	6 0.004-15	/	/	:	Wagemann <i>et al.</i> , 1990
		Canadian western Arctic	77	108 1-464	/	9 0.5-27	39 15-88	75 3-235	45 3-140	112 43-185	Wagemann <i>et al.</i> , 1996
		Canadian eastern Arctic	73	41 5-154	/	26 0.03-103	90 0.3-314	21 1.5-91	77 3-1324	115 41-361	Wagemann <i>et al.</i> , 1996
Kogiidae	<i>Kogia breviceps</i>	Argentina	1	47	/	30	1650	/	40	652	Marcovecchio <i>et al.</i> , 1990
Physeteridae	<i>Physeter macrocephalus</i>	North Sea	6	41 9-61	5% 2-8%	82 52-175	258 133-426	18 6-43	8 5-12	104 90-125	Bouqueneau <i>et al.</i> , 1997 <sup>b</sup> Holsbeek <i>et al.</i> , 1999
Phocoenidae	<i>Phocoena phocoena</i>	North Sea	5	170 1-504	37%	/	/	/	/	/	Joiris <i>et al.</i> , 1991
		Baltic sea	4	/	/	0.3 0.3-0.4	1.5 0.2-3	/	24 18-6	120 96-144	Szefer <i>et al.</i> , 1994
		West Greenland	44	16 2-80	/	13 0.2-45	53 0.4-290	11 2-36	48 20-200	200 145-370	Paludan-Müller <i>et al.</i> , 1993
	<i>Phocoenoides dalli</i>	Nothwestern Pacific	3	6 (n=1; fœtus)	/	/ 0-84	/ 0-136	/	/ 20-329	/ 110-186	Fujise <i>et al.</i> , 1988

<sup>1</sup> Median

When the range was not available, standard deviation was used (all the data are expressed in dry weight assuming a mean water content of 75% of the tissues).

potentially toxic even at low concentrations. Nickel and copper hazards to wildlife have been extensively reviewed by Eisler (1997; 1998). When considering marine mammals, there are limited data about heavy metals, except for mercury. Chromium, nickel and lead concentrations are generally low, rarely exceeding a few  $\mu\text{g.g}^{-1}$  dry weight (dw) in marine mammal tissues. No meaningful assessment of their toxicity in marine mammals can be made as yet (Law, 1996). However, investigations carried out on a ringed seal population (*Pusa hispida saimensis*) from Finland showed a clear connection between stillbirth of the pups and nickel concentrations in the air (H  varinen and Sipil  , 1984). These authors have underlined the considerable nickel input in the environment from industrial activity in that particular area. On the other hand, zinc, copper, cadmium and mercury concentrations often exceed several tens  $\mu\text{g.g}^{-1}$  dw and so will be discussed in particular as well as their levels in the different marine mammal groups, detoxification mechanisms, potential hazards and ecological implications.

## 2. FACTORS AFFECTING HEAVY METAL CONCENTRATIONS IN MARINE MAMMALS

Some reviews about heavy metal contamination of marine mammals have been published (Wagemann and Muir, 1984; Thompson, 1990; Kemper *et al.*, 1994; Law, 1994; 1996). Tables 1 to 5 present some selected references. It appears that metal concentrations vary greatly within marine mammals, especially for non-essential metals such as cadmium and mercury (table 1).

**Table 1:** Examples of maximum and minimum metal concentrations for marine mammals

Metal	Minimum	Maximum
Hg	0.2 muscle <i>Pusa hispida</i> Western Arctic Wagemann <i>et al.</i> , 1996	13156 liver <i>Tursiops truncatus</i> Mediterranean Sea Leonzio <i>et al.</i> , 1992
Cd	0.007 muscle <i>Pusa hispida</i> (<1y.) Northwest Greenland Dietz <i>et al.</i> , 1998	2324 kidney <i>Pusa hispida</i> (8y.) Northwest Greenland Dietz <i>et al.</i> , 1998
Zn	2 blubber <i>Leptonychotes weddellii</i> (13 y.) Antarctic Yamamoto <i>et al.</i> , 1987	4183 liver <i>Dugong dugon</i> (>30 y.) Australia Denton <i>et al.</i> , 1980
Cu	0.4 blood <i>Leptonychotes weddellii</i> (13 y.) Antarctic Yamamoto <i>et al.</i> , 1987	600 liver <i>Tursiops truncatus</i> Argentina Marcovecchio <i>et al.</i> , 1990

When available, the age of the animals is given (all the data are expressed in  $\mu\text{g.g}^{-1}$  dry weight, assuming water content of 75% of the tissue).

The large variation of these data (table 1) illustrates the numerous physiological and ecological factors that might affect heavy metal contamination: geographic location, diet, age, sex, the tissues considered and metabolic rates. Information concerning metabolic rates is largely unavailable for the different marine mammals species so will not be discussed here. However it must be kept in mind that ingestion and assimilation rates differ between all the marine mammal species in relation with their weight and their migration or physiological status (fasting).

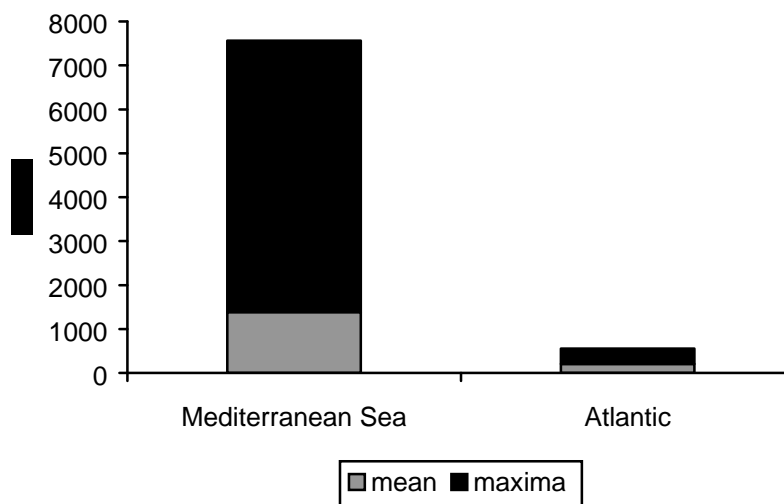
### 2.1. Geographic location

Heavy metal contamination sources can be both anthropogenic and natural and, distinguishing between the two can be very difficult. The natural background component of the input of heavy metals in marine ecosystems may be as important as the anthropogenic one, and, in some areas, it appears to be the major source. This is important because it emphasises that marine mammals have been exposed to heavy metals long before the development of human activities. It is *e.g.* the case for the Mediterranean Sea and the Arctic, which are known for their high natural metal levels: Hg in the Mediterranean Sea and Cd in the Arctic.

The mercury levels measured in dolphins from the Mediterranean Sea are higher than those encountered in

dolphins from Pacific coasts of Japan or Northeast Atlantic (figure 1) (André *et al.*, 1991<sup>a</sup>; Honda *et al.*, 1983; Leonzio *et al.*, 1992). According to André *et al.* (1991<sup>b</sup>), the origin of high mercury levels observed in Mediterranean dolphins is certainly natural because of the large natural sources present in the Mediterranean basin.

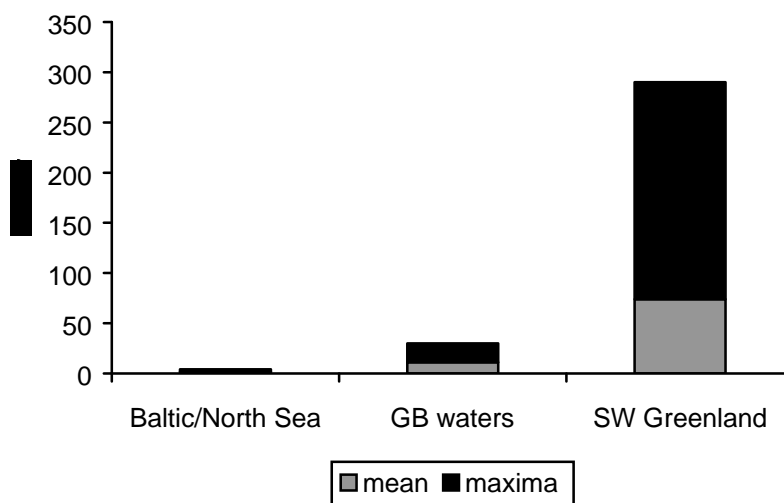
**Figure 1:** Mercury levels in livers from striped dolphins (*Stenella coeruleoalba*) from Northeast Atlantic and Mediterranean Sea (after André *et al.*, 1991<sup>a</sup>; 1991<sup>b</sup>)



Concentrations are expressed in dry weight assuming mean water content of 75% of the tissues.

The current state of knowledge of concentrations, spatial and temporal trends of contaminants including heavy metals have been extensively described in the Arctic (Muir *et al.*, 1992; Dietz, 1996; AMAP, 1998). It seems that cadmium concentrations have always been high in the Greenland Arctic regions as indicated by the lack of obvious temporal trends in sediment cores, as well as historic hair samples from the 15<sup>th</sup> century from both seals and Inuits (Dietz *et al.*, 1998). Johansen *et al.* (1980) first reported that cadmium levels in tissues of ringed seals from Greenland were higher than previously reported for seals from the North Sea. Cadmium seems to accumulate to higher levels in seals living in unpolluted Arctic waters than those taking their prey in the North Sea area, which receives large inputs of pollutants. A similar observation can be made for porpoises (figure 2).

**Figure 2:** Cadmium levels in kidney from porpoises (*Phocoena phocoena*) from Baltic and North Seas, Great Britain waters and Southwestern Greenland (Harms *et al.*, 1978; Falconer *et al.*, 1983; Paludan-Müller *et al.*, 1993).



Concentrations are expressed in dry weight assuming mean water content of 75% of the tissues.

Wagemann *et al.* (1996) have investigated the heavy metal distribution in belugas (*Delphinapterus leucas*), narwhals (*Monodon monoceros*) and ringed seals within the Arctic region. Mean mercury concentrations in the livers of belugas and ringed seals were significantly higher in the Western Arctic than in the Eastern Arctic. This comparison was not possible for narwhals, as they are not found in the Western Canadian Arctic. On the contrary, cadmium as well as zinc and copper concentrations in tissues (liver and kidney) of belugas and ringed seals were higher in the Eastern than in the Western Arctic. These differences in metal levels between marine mammals of the Eastern and Western Arctic corresponded to the different geological settings and sediment of these two regions (Wagemann *et al.*, 1995; 1996).

## 2.2. Routes of entry

There exist several different routes of entry of heavy metals in marine mammals: uptake from the atmosphere through the lungs, absorption through the skin, across the placenta before birth, via milk through lactating, ingestion of sea water and ingestion of food. Nevertheless, the major route of heavy metal contamination for marine mammals seems to be via feeding (André *et al.*, 1990<sup>a,b</sup>; Augier, 1993<sup>c</sup>; Law, 1996). Considering that, the following discussion will mainly refer to the diet (including suckling) and its influence on metal uptake.

Obviously, mysticetes are less contaminated by heavy metals than odontocetes and pinnipeds (which are located at higher trophic levels in the marine food web). Cadmium seems to be transferred to the highest trophic levels of the marine food chain mainly by molluscs, particularly cephalopods, which concentrate cadmium in their viscera (Honda and Tatsukawa, 1983; Bouquegneau and Joiris, 1988; Miles and Hills, 1994). Long-finned pilot whales (*Globicephala melas*) for example are known to concentrate cadmium in relation with a preferential cephalopod diet. Elevated levels of cadmium in Pacific walrus (*Odobenus rosmarus divergens*) and northern fur seals (*Callorhinus ursinus*) have been reported in a population of the Bering Sea, a remote area away from industrial activity (Miles and Hills, 1994). In an attempt to find out which prey may transfer cadmium to walrus, the most common preys (mainly bivalves) found in the stomach contents were analysed for their metal concentrations. Amongst the bivalves analysed, *Mya sp.* showed the highest cadmium content suggesting that this prey could be a cadmium transmitter for walrus (Miles and Hills, 1994). Wagemann and Stewart (1994) have studied heavy metal concentrations of walrus (*Odobenus rosmarus rosmarus*) from the Eastern Canadian Arctic in relation with their food, mostly bivalves (*Mya sp.* and *Serripes sp.*). These authors showed that bivalves could also be a source of lead for walrus, judging the correspondence between the high levels of Pb in both bivalves and walrus (Wagemann and Stewart, 1994).

However, some high cadmium levels have been reported in some marine mammals that do not eat cadmium-contaminated prey (Denton *et al.*, 1980). Trace metals have been determined in dugong, *Dugong dugon*, which mostly feeds on seagrasses and algae. High cadmium and zinc concentrations have been detected in their liver and kidney. Denton *et al.* (1980) have reported renal cadmium and hepatic zinc concentrations reaching 309

$\mu\text{g.g}^{-1}$  and  $4183 \mu\text{g.g}^{-1}$  dry weight respectively while low levels of these metals are found in the seagrass analysed. The authors suggested that the low levels of Cu in seagrasses may influence intestinal absorption of Zn and Cd in the dugong. Higher than normal Zn and Cd absorption through the intestinal tract occurs when dietary Cu intake is deficient, due to competition between the metals for available binding sites on carrier proteins within the intestine (Denton *et al.*, 1980). Unlike dugongs of coastal Australia which display a copper deficiency due to seagrass diet, Florida manatees (*Trichechus manatus*) are considered to be facing an opposite problem: Cu concentrations in the livers of Florida manatees were significantly elevated in areas of high herbicidal copper usage (O'Shea *et al.*, 1984). Manatees might be inefficient at maintaining copper homeostasis in the face of dietary excess. The death of a captive dugong was attributed to exposure to copper sulphate added to its tank as an algicide (Oke, 1967).

The position of top predators in the food web influences mercury levels in marine mammals as this highly toxic metal is biomagnified, when available as methylmercury, through the food web (Bouquegneau and Joiris, 1988). Diets, and especially those of marine mammals relying on fishes, are responsible for mercury contamination (Svensson *et al.*, 1992; Nakagawa *et al.*, 1997). Much higher mercury concentrations have been reported in minke whales, (*Balaenoptera acutorostrata*), from Greenland (Hansen *et al.*, 1990) compared to Antarctic minke whales (*Balaenoptera bonaerensis*), which are several times less contaminated (Honda *et al.*, 1986; 1987). Hansen *et al.* (1990) attributed the lower mercury concentrations found in tissues of Antarctic minke whales to differences in trophic levels and subsequently in the mercury content of the food items: Northern minke whales feed mainly on fish while Southern minke whales feed on krill. Indeed, the food web in the Antarctic ecosystem is rather simple, as the major food component is the Antarctic krill. The low trophic position of the Antarctic minke whale is reflected in the very low bioconcentration factor of Hg in this animal.

### 2.3. Age

Cadmium and mercury accumulated strongly with age in most marine mammals tissues analysed (*e.g.*: Hamanaka, 1982; Honda and Tatsukawa, 1981; 1983; Honda *et al.*, 1983; Augier *et al.*, 1993b). This increase is enhanced in the liver in which the excretion rate might be extremely low due to the fossilisation of mercury under a detoxified form (see below). However, some exceptions have been reported in the literature. Honda *et al.* (1986; 1987) have studied the heavy metal distribution in Antarctic minke whales, and compared it to their food habit and age. The age of these whales varies between 1 and 45 years. They found maximum concentrations of Cd and Hg in the livers of 20 year old minke whales. Both concentrations increase with age until about 20 years and thereafter decrease. Because there is no evidence that half-lives of cadmium and mercury change with age, Honda *et al.* (1986; 1987) suggested a higher food intake for the younger animals compared to older. They also suggested that these changes may be due to the significant decrease in stocks of blue whales (*Balaenoptera musculus*), and fin whales, (*Balaenoptera physalus*). Both species used to occupy ecological niches similar to those of the minke whales in the Antarctic marine ecosystems. The young minke whales would be less exposed to feeding competition from blue and fin whales, than the older ones. Accumulations of toxic metals such as Cd or Hg may also have been influenced by this ecosystem disruption (Honda *et al.*, 1986; 1987).

High copper and zinc concentrations have also been observed in very young animals and neonates (*e.g.*: Julshamn *et al.*, 1987; Wagemann *et al.*, 1988; Caurant *et al.*, 1994). These essential elements are known to increase in tissues undergoing rapid development and differentiation (Baer and Thomas, 1991). It has been suggested that these higher levels encountered in young might reflect a specific requirement in newborns or a very low excretion rate of these metals by the foetus (Wagemann *et al.*, 1998).

### 2.4. Sex

Reproductive activities such as pregnancy, parturition and lactation can modify the metal levels. Several studies have demonstrated that metal transfer from females to pups occur through the placenta or lactation: Honda *et al.* (1987) reported a hepatic Fe, Co, Pb and Ni transfer from mother to pup. As a consequence, these metal concentrations decrease in the mature female with progress of gestation.

Canella and Kitchener (1992) found significantly lower levels of mercury in pregnant and lactating sperm whales (*Physeter macrocephalus*) when compared with non-breeding females. They suggested that this may be due to hormonal changes or stress causing the redistribution of mercury in body tissues.

## 2.5. Distribution within organs

The pattern of site distribution of metals within the organism is tissue and metal specific. For example, mercury is mostly concentrated in the liver, with kidney and muscle having successively lower levels. On the contrary, the highest cadmium concentrations are usually encountered in kidney due to the presence of metal binding proteins. This pattern prevails in most marine mammals concentrations (Wagemann and Muir, 1984).

Yamamoto *et al.* (1987) have studied the distribution of heavy metals in the whole organism of three Weddell seals (*Leptonychotes weddelli*) from the Antarctic. These authors estimated the whole metal body burden, which was calculated from the weight of the different tissues and their respective concentrations. If whole body burdens of metal are estimated for an adult Weddell seal, zinc is mostly located in muscles and in bones, copper in muscles and liver, Hg in liver and muscles and Cd in liver and kidney. The apparent contrast between the low concentration in Hg generally measured in marine mammals muscles and the high burden encountered is due to the high muscle mass of these animals. The redistribution of mercury from highly contaminated organs such as liver or kidney through muscles seems to be a protection mechanism against mercury toxicity (Cuvin-Aralar and Furness, 1991).

Skin contains generally low Hg concentrations (Yamamoto *et al.*, 1987). However, the skin of marine mammals is not a homogenous tissue. It consists of four distinct layers in which mercury increases progressively outwards with a concentration of  $6 \mu\text{g.g}^{-1}$  (estimation in dry weight) in the outermost layer of Arctic belugas and narwhals. During the process of moulting, the last and the underlying are shed and approximately 20% of the total mercury in the skin is lost annually in belugas and 14 % by narwhals (Wagemann *et al.*, 1996).

## 3. TOXICITY OF MERCURY AND CADMIUM

As quoted above, the accumulation through the food chain is a major risk for top predators. The accumulation of chemical substances may result in toxic concentrations in organisms (secondary poisoning) even if the concentration in the environment remains below the threshold level for direct toxicity (Nendza *et al.*, 1997). The finding of high concentrations of metals such as cadmium or mercury has raised the question about their toxicity.

Reliable toxicity data for predating marine mammals are scarce. Instead, threshold levels are often extrapolated from terrestrial species, *i.e.* interspecies correlations are assumed to hold for rats or captive seals. The validity of these extrapolations is highly questionable and can only be justified by the current lack of better data and by ethic considerations. Indeed, potential effects of toxic metals cannot be tested in free-living animals because experimental manipulations are undesirable. *In vitro* experimentations or systematic post-mortem investigations to establish the disease status of contaminated animals in a relatively large number of individuals from the same species are a complementary and valuable alternative way to understand the numerous processes involved.

### 3.1. Mercury toxicity

Mercury exists in several interchangeable forms in the biosphere (Kaiser and Tölg, 1980), but the mercury accumulation through the food web mainly occurs under an organic form, methylmercury (MeHg), as a result of its lipid solubility and preferential assimilation during zooplankton grazing (Mason *et al.*, 1995). Above zooplankton, organic mercury is biomagnified along the food chain up to marine mammals.

Total mercury concentration is a poor indicator of toxic effects, as organic mercury compounds seem to be considerably more toxic to animals than inorganic mercury. The biological and toxicological activity of mercury depends on the form that is taken up, the route of entry in the body (skin, inhalation or ingestion), and on the extent to which mercury is absorbed (Kaiser and Tölg, 1980). Wolfe *et al.* (1998) have recently reviewed the toxicity on mercury on wildlife.

In mammals, methylmercury toxicity is primarily manifested as central nervous system damage including sensory and motor deficits and behavioural impairment. Animals become anorexic and lethargic. Methylmercury is easily transferred across the placenta (Wagemann *et al.*, 1988) and thus concentrates in the foetal brain (Wolfe *et al.*, 1998). This reproductive effect ranges from development alterations to foetal death.

Methylmercury is also absorbed by the gastrointestinal tract while inorganic salts of Hg are less readily absorbed. It is mainly as methylated form that mercury is absorbed from fish, since almost all the mercury present in fish is methylated (Svensson *et al.*, 1992).

Experimental seal intoxication has led the animals to lethargy, weight loss and finally death (Ronald and

Tessaro, 1977). Four harp seals (*Pagophilus groenlandicus*) were intoxicated with methylmercury by a daily oral intake. Two seals were fed with mercury doses of 0.25 mg/kg of body weight per day for 60 and 90 days. These two seals did not show abnormal blood concentrations but exhibited a reduction in appetite and consequent weight loss. Two others seals fed 25 mg/kg of body weight per day died on day 20 and day 26 of exposure. The measurements of blood parameters indicated toxic hepatitis, uremia and renal failure. These pathologies have been related to high accumulation of mercury in these tissues. The liver concentrations reached more than 500 µg/g dry weight after the death. Almost 90% of the mercury analysed in the liver were methylmercury. No detoxification mechanisms were described in this case.

However, this experimental study did not reproduce the real daily food intake of marine mammals in the wild. Nigro and Leonzio (1993) have calculated a mean daily food for small cetaceans of approximately 3 kg of fishes and cephalopods with an average mercury concentration of 0.3 mg/kg fresh weight, the mean dietary mercury intake for an adult specimen can be estimated at 0.9 mg Hg per day for the whole animal. It is quite far from the 25 mg/kg of body weight and per day administrated in gel caps to those seals. Another feature that might explain the absence of detoxification is the absence of selenium added to the food. In the wild, if fish are an source of exposure to mercury, they are also a source of selenium (Svensson *et al.*, 1992) and in marine mammals demethylation mechanisms occur in the presence of selenium (see below). In this experimental study, the lack of additional selenium in the diet was probably the limiting factor to detoxification.

Very few studies have tried to link metal concentrations measured in free ranging marine mammals and health status (Hyyvarinen and Sipilä, 1984; Rawson *et al.*, 1993; 1995; Siebert *et al.*, 1999; Bennet *et al.*, 2001). Only one case of Hg toxicose has been reported by Helminen *et al.* (1968): the ringed seal suspected of Hg intoxication was from an area of heavy industrial mercury dumping.

Chronic mercury accumulation was associated with liver abnormalities observed in stranded bottlenose dolphins from the Atlantic. Large deposits of a brown pigment, identified as lipofuscin, in the portal areas of the liver were observed in the livers of nine animals with high hepatic mercury levels (> 60 µg/g fresh weight). Analytical electron microscopy carried out on these pigments demonstrated that HgSe was the predominant material (Rawson *et al.*, 1995). Lipofuscin is believed to be derived from damaged subcellular membranes. This pigment accumulation strongly correlates with mercury concentrations. Mercury would have inhibited the activity of lysosomal digestive enzymes and therefore, reduced degradation of proteins. This has led to excessive accumulation of lipofuscin within cells and finally cell death. (Rawson *et al.*, 1993).

More recently, Siebert *et al.* (1999) examined the possible relationship between Hg tissue concentrations and disease in harbour porpoises from the German waters of the North and Baltic Seas. Higher mercury content has been measured in organs of the harbour porpoises from the North Sea compared to those of the Baltic Sea, indicating that mercury is a more important threat for animals of the North Sea than for Baltic Sea. High mercury concentrations were associated with prevalence of parasitic infection and pneumonia.

Bennet *et al.* (2001) have also used this indirect approach to investigate the prediction that increased exposure to toxic metals results in lowered resistance to infectious disease in harbour porpoises from the coasts of England and Wales. Mean liver concentrations of Hg, Se, Hg:Se ratio, and Zn were significantly higher in the porpoises that died of infectious diseases (parasitic, bacterial, fungal and viral pathogens such as pneumonia), compared to porpoises that died from physical trauma (most frequently entrapment in fishing gear). Liver concentrations of lead, cadmium, copper, and chromium did not differ between the two groups.

In some cases, balances between elements seem to be more important than the absolute concentration when the possibility of toxic effects is considered. High premature birth rates have been observed and studied between 1968 and 1972 in the Californian Sea lion (*Zalophus californianus*) from the southern California Channel Island rookeries (Martin *et al.*, 1976). These premature pups were ataxic, had difficulties in breathing and died shortly after birth. Heavy metals were analysed and compared between normal and premature pups and between their respective mothers. The results revealed that severe imbalance in the Hg:Se:Br occurred in the livers of the abnormal mothers. The absolute concentrations seemed not to be involved in this case as Hg, Se, and Br were in higher concentrations in the livers of normal mothers compared to abnormal mothers. This suggests that Se:Hg balance is a very complex phenomenon and might be more important for general health status than absolute concentrations.

Some *in vitro* studies have also been realised to evaluate the potential hazard of mercury in marine mammals. Freeman and Sangaland (1977) demonstrated that methylmercury alters the *in vitro* synthesis of steroid hormones which play an important role in reproduction. Genetic effects of methylmercury on lymphocytes of one bottlenose dolphin (*Tursiops truncatus*) have also been evaluated *in vitro* by Betti and Nigro (1996).

Lymphocytes were isolated from blood obtained from a 15 year old dolphin (Adriatic Sea). Methylmercury induces DNA single-strand breaks and cytotoxicity in a dose-dependent manner. The doses of MeHg used in this study are likely to be in the range of concentrations (between 1 and 10  $\mu\text{g/ml}$ ) naturally occurring in the blood of wild dolphins found in the Mediterranean Sea (Betti and Nigro, 1996). It appears that dolphin lymphocytes have a greater resistance both to the genotoxic and cytotoxic effects of MeHg when compared to human or rat cells. This feature can be interpreted as an adaptation acquired by dolphins to counter the methylmercury exposure.

### 3.2. Cadmium toxicity

Cadmium is regarded as one of the most toxic metals. High dietary concentrations of cadmium in humans can lead to well known heavy skeletal deformities ('itai-itai' disease), kidney lesions (mainly on the proximal tubules) usually preceeding lung damages, dysfunction of cardiovascular and hematopoietic system as well as carcinogenic, mutagenic and teratogenic effects (Förstner, 1980; Lamphère *et al.*, 1984; Jonnalagadda and Prasada Rao, 1993). Effect of cadmium on marine ecosystems has been recently reviewed (AMAP, 1998). The renal concentrations can reach levels as high as 2000  $\mu\text{g.g}^{-1}$  dry weight in some Arctic ringed seals (Dietz *et al.*, 1998). This is much higher than the critical concentrations of approximately 800  $\mu\text{g.g}^{-1}$  dry weight (200  $\mu\text{g.g}^{-1}$  fresh weight) associated with kidney damage in mammals including humans (WHO, 1992). Moreover, following Elinder and Järup (1996), this critical concentration has been largely overestimated as cadmium-induced renal dysfunctions have been displayed within order of kidney cortex concentrations of 200  $\mu\text{g.g}^{-1}$  dry weight (50  $\mu\text{g.g}^{-1}$  fresh weight). For comparison, in human adults, the renal cadmium concentration amongst non-smokers is less than 5  $\mu\text{g.g}^{-1}$  fresh weight (Pesch *et al.*, 1989). No obvious cadmium toxic effect has been registered till now in marine mammals despite the high levels encountered in several species suggesting highly efficient detoxification mechanisms (Dietz *et al.*, 1998).

## 4. DETOXIFICATION MECHANISMS

The exposure of marine mammals to heavy metals has occurred throughout their evolutionary history, during which they have developed mechanisms either to control the internal concentration of certain elements or to mitigate their toxic effects. The most obvious case is the one of mercury in dolphins. Compared to other terminal consumers like tunas or seabirds, some marine mammals accumulate much higher levels of mercury with biomagnification factors in respect to prey of 500 in dolphins compared to 30 *e.g.* in predatory fish (Leonzio, 1996). This can be explained by physiological differences not only in the involved uptake and release, but also - and sometimes mainly - in detoxification processes.

### 4.1. Mercury detoxification

Wagemann and Muir (1984) found mercury and selenium concentrations reaching up to 510  $\mu\text{g.g}^{-1}$  fresh weight (approximately 2000  $\mu\text{g.g}^{-1}$  dry weight). Despite such extremely high values, the animals did not show any overt signs of mercury or selenium poisoning because the presence of the two elements together provided protection to the animal. Many studies have demonstrated the mutual antagonism between mercury and selenium (Pelletier, 1985; Cuvin-Aralar and Furness, 1991). The mutual antagonism between these two elements has become one of the strongest and most general examples of interactions between heavy metals. This phenomenon occurs throughout the animal kingdom from oysters, shrimps to marine mammals and human beings. Koeman *et al.* (1973; 1975) first reported the strong correlation between mercury and selenium in livers of marine mammal species. A molar ratio Hg:Se of approximately 1 has been observed suggesting mercury detoxification mechanisms in presence of selenium.

Different forms of mercury coexist in the environment. Methylmercury is known to be one of the most toxic. Mercury is transferred up to marine mammals in a methylated form. However, very small amounts of methylmercury are generally found in the liver of marine mammals: less than 10% of mercury is present in a methylated form in the livers of adult marine mammals. Other mercury occurs also in an inorganic form (Wagemann *et al.*, 1998) which implies that a demethylation process occurs (Joiris *et al.*, 1991). The fate of this inorganic mercury has been mainly elucidated by histological studies carried out in livers from specimens of Cuvier's beaked whale (*Ziphius cavirostris*) and bottlenose dolphins (Martoja and Viale, 1977; Martoja and Berry, 1980). These authors first observed granules composed of mercuric selenide ( $\text{HgSe}$ ). Successively, similar granules were also described in the striped dolphin (*Stenella coeruleoalba*). Mercury and selenium occurred as dense intracellular granules, located mainly in the liver macrophages, the Kupfer cells, and in the proximal tubules of the kidney. These granules appear as spherical or polygonal particles ranging from 15 to 80 Å (Augier *et al.*, 1993<sup>a</sup>; Nigro and Leonzio, 1993; Nigro and Leonzio, 1996).

More recently, Rawson *et al.* (1995) found HgSe crystals in both the liver and respiratory system of the bottlenose dolphin and short-finned pilot whale and reported HgSe in the lung and hilar lymph nodes associated with soot particles. In both the liver and hepatic lymph nodes, these crystals were small, averaging 50 Å. In the lung and hilar lymph nodes, the crystals were much larger, measuring 250-500 Å. Abundant carbon was present in the hilar nodes while only very small amounts were found in the hepatic nodes and in the liver. These findings suggest an alimentary and a respiratory entry for Hg in cetaceans: Hg in the liver is likely to be trophically acquired, passing through the gastrointestinal tract and carried to the liver by the way of portal veins. In the liver, it may be converted into HgSe accumulating as an end product (Martoja and Berry, 1980). In animals producing large amount of HgSe, some of this may be carried to the hepatic lymph nodes and even to the spleen (Rawson *et al.*, 1995). HgSe in the lungs and the hilar nodes appears to be closely associated with carbon suggesting an atmospheric association between these elements. Indeed, mercury and selenium pollution is largely attributed to the burning of fossil fuel or waste incinerations and these elements tend to aggregate as particles (Rawson *et al.*, 1995). However, *in vivo* precipitation of HgSe into the surfaces of inhaled soot particles cannot be ruled out and further investigations are needed to understand this lung accumulation process. These mercuric selenide granules seem to be the last step of a very efficient detoxification mechanism leading to high but non-toxic concentrations in the organs. The 1 molar ratio has been generally observed in marine mammals. However, the Hg:Se molar ratio found in the liver can vary within the range of 0.2 (Hansen *et al.*, 1990) to 2.49 (Caurant *et al.*, 1996).

Palmisano *et al.* (1995) have explained this variation: Hg:Se molar ratio of approximately 1 has been observed in striped dolphin livers only after a certain threshold in the total Hg concentration (approximately 100 µg.g<sup>-1</sup> fresh weight) has been exceeded. Palmisano *et al.* (1995) have proposed a two-stage mechanism for the demethylation and accumulation process. At low Hg levels (first stage) the metal is retained mainly in its methylated form. At higher Hg levels (second stage) demethylation, with a concurrent accumulation of Se, seems to be the prevailing mechanism. Moreover, above the threshold only a small fraction of mercury is present in a labile form as Hg<sup>++</sup> and MeHg<sup>+</sup> bound to the cysteine residue. These authors have determined that 63% of the total mercury analysed in the liver of one dolphin is involved in the formation of a very insoluble selenocompounds, certainly present as HgSe (tiemannite) but in addition, mercury can be involved in the formation of other seleno-compounds as Hg-selenoproteins. This hypothesis of a threshold has also been suggested in other studies dealing with mercury speciation (Sanpera *et al.*, 1993; Caurant *et al.*, 1996). Sanpera *et al.* (1993) have found no decrease in the organic mercury fraction with increasing total mercury concentration in the livers of fin whales (*Balaenoptera physalus*). On the contrary, Caurant *et al.* (1996) found in livers of longfinned pilot whales a decreasing correlation between organic mercury and total mercury. Organic mercury was lower than 2% when the total mercury concentration was higher than 100 µg.g<sup>-1</sup> fresh weight (400 µg.g<sup>-1</sup> dry weight). Only a small fraction (<1%) of total mercury is bound to heat-stable compounds (which include the metallothioneins). These heat-stable proteins are able to bind bivalent metals and are believed to participate in heavy metal detoxification processes (see above). More than 90% of the mercury was in the insoluble fraction except for two animals. In these two individuals, insoluble mercury in the liver was < 90% and 14% and 35% of mercury were bound to metallothioneins. These individuals exhibited total mercury concentrations <50 µg.g<sup>-1</sup> fresh weight (200 µg.g<sup>-1</sup> dry weight) in the livers. When total mercury was higher than this value, the percentage bound to metallothioneins was low always <1% whatever the total mercury concentration (Caurant *et al.*, 1996).

In some species, mercury concentrations can be low during the entire life span. Fin whales feed at the bottom of the trophic web (Sanpera *et al.*, 1994) and their mercury levels stay relatively low. In their livers, the mean ratio of organic/total mercury is about 40%. This result seems very high compared to other data (see table 6). The authors suggest that demethylation is carried out at a constant rate, probably because concentrations remain low throughout their life span, between 50 and 500 µg.g<sup>-1</sup> dry weight (Sanpera *et al.*, 1994). It seems in this case that the specific threshold has not been reached and so high percentages of organic mercury are observed in the livers.

Young marine mammals can display a high percentage of methylmercury (table 6) suggesting that they do not reach a specific threshold (Palmisano *et al.*, 1995). A second hypothesis is that young individuals are still unable to demethylate mercury efficiently (Caurant *et al.*, 1996).

**Table 6:** Comparison of mean percentage (%) methylmercury to total mercury in suckling marine mammals compared to adults

Species	Pups	Adults	Sources
<i>Pagophilus groenlandicus</i>	70	14	Wagemman <i>et al.</i> , 1988
<i>Stenella coeruleoalba</i>	45	2.5	Itano <i>et al.</i> , 1984
<i>Globicephala melas</i>	55	17	Caurant <i>et al.</i> , 1996

## 4.2. Cadmium detoxification

As quoted above, the concentrations of cadmium in kidneys of marine mammals can reach levels more than two times the critical concentrations of approximately  $800 \mu\text{g}\cdot\text{g}^{-1}$  dry weight ( $200 \mu\text{g/g}$  fresh weight) associated with kidney damage in terrestrial mammals including humans (WHO, 1992). This raises questions about the health status of such heavily cadmium contaminated marine mammals. Dietz *et al.* (1998) have compared low and high cadmium contaminated kidneys from ringed seals from Northwest Greenland in an attempt to do macroscopic and light microscopic examinations. No differences in renal morphology could be observed between experimental groups. These investigations indicate that marine mammals appear to be able to maintain considerable concentrations of cadmium without showing renal damage. Dietz *et al.* (1998) have therefore postulated that ringed seals are adapted to the naturally high cadmium levels of the Greenland Arctic regions.

### 4.2.1. Role of metallothioneins

Marine mammals might mitigate the toxic effects of cadmium through binding to metallothioneins (reviewed by Das *et al.*, 2000). The role of metallothioneins in cadmium detoxification has been often raised since the first isolation of this protein in horse kidney cortex by Margoshes and Vallee (1957). The presence of these low-molecular weight proteins has been further demonstrated in many organisms ranging from blue green algae to primates (Kägi, 1991). These soluble and heat-stable proteins are found in the cytosolic fraction of the tissues. They are quite unique as they are characterised by a high cysteine content, divalent ion inducibility, such as  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , and high affinity in the binding of these cations (Kägi, 1991; Roesijadi, 1992; 1996). Metallothioneins primary function is the homeostasis of essential heavy metals, specifically zinc and copper. Zinc-thionein levels increase in tissues undergoing rapid development and differentiation such as the neonatal rat-liver. Zinc-thioneins are also able to transfer zinc to metal dependent enzymes in case of high metabolic demand (Baer and Thomas, 1991). As a result of this capacity to bind cations, metallothioneins are able to bind non-essential metals such as  $\text{Cd}^{2+}$ ,  $\text{Ag}^{+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ . In this way, these proteins reduce the bioavailability of these ions and consequently their toxicity. Metallothioneins are produced by several tissues but liver and kidney cells are the more potent producers and consequently preferential accumulation of heavy metals occurs in these tissues (Kägi, 1991). According to Cosson *et al.* (1991), their participation in detoxification mechanisms would be due to nothing more than fortuitous interactions of foreign cations with the normal homeostasis mechanisms for Zn and perhaps Cu. However, these proteins are able to bind important amounts of cadmium and so prevent the cellular damage in the organism (Viarengo, 1989; Roesijadi, 1992).

Metallothioneins have already been characterized from livers and kidneys of different marine mammals such as grey seals (*Halichoerus grypus*) and northern fur seals (Olafson and Thompson, 1974), California Sea lions (Ridlington *et al.*, 1981), striped dolphins (Kwohn *et al.*, 1986), harbour seals (Mochizuki *et al.*, 1985; Tohyama *et al.*, 1986), narwhals (Wagemann and Hobden, 1986) and sperm whales (Bouquegneau *et al.*, 1997; Holsbeek *et al.*, 1998). The role of metallothioneins has been recently reviewed by Das *et al.* (2000).

A study performed on harbour seals caught on Japanese coasts showed that metallothionein concentrations are significantly correlated with the level of Cd and Cu in the livers and Cd, Zn, Cu and inorganic Hg in the kidneys (Tohyama *et al.*, 1986). Correlations were also found with age.

**Table 7:** Cadmium speciation in the whole tissue, the cytosolic fraction and metallothioneins

Species	Tissues	Cd ( $\mu\text{g}\cdot\text{g}^{-1}\text{dw}$ )	(%) of Cd in cytosolic fraction	% Cd bound to cytosolic metallothioneins	Sources
<i>Stenella coeruleoalba</i>	kidney	87	58	98	Khwon <i>et al.</i> , 1986
<i>Zalophus californianus</i>	kidney liver	37 <dl	68 <dl	nd <dl	Ridlington <i>et al.</i> , 1981
<i>Globicephala melas</i>	kidney liver	548 312	nd nd	54 51	Amiard-Triquet and Caurant, 1997
<i>Physeter macrocephalus</i>	liver	50	90	nd	Ridlington <i>et al.</i> , 1981
		95	49	9	Bouquegneau <i>et al.</i> , 1997
	kidney	258	83	40	Holsbeek <i>et al.</i> , 1999
<i>Monodon monoceros</i>	kidney	332	92	72	Wagemann <i>et al.</i> , 1984; 1986
	liver	176	88	77	

Cd concentrations are estimated in  $\mu\text{g/g}$  dry weight; nd: not determined, dl: detection limit

The percentage of the cytosolic cadmium bound to metallothioneins varies from 9 to almost 100% (table 7). It is interesting to note that this 9 % value has been measured in highly debilitated sperm whales found stranded on the Belgian coast (Bouquegneau *et al.*, 1997; Holsbeek *et al.*, 1998). This implies that cadmium did not occur in a detoxified form. These animals were seriously debilitated as indicated by their reduced blubber thickness and body weight (Jauniaux *et al.*, 1998). Cadmium which is known to induce debilitation in mammals can be considered as one of the factors responsible for the debilitation of these animals, a condition which could have favoured their stranding, in addition to stress and starvation (Bouquegneau *et al.*, 1997).

Other results described in the table 7 have been obtained from captured and not stranded animals. More than 80 % of the hepatic cadmium and 90 % of the renal cadmium is located in the cytosolic fraction of the tissues of the narwhal in which metallothioneins are located. Almost all this cytosolic cadmium was bound to these proteins indicating that the metallothionein mechanism was not saturated (Wagemann *et al.*, 1984; 1986). This could be an adaptation of this Arctic species to the high cadmium concentrations present in its environment. More data on cadmium speciation in marine mammals should be collected to get a better comprehension of the precise role of metallothioneins in detoxification processes. It has been suggested that cadmium toxicity occurs when available metallothioneins are insufficient to bind all the cadmium. Recent experiments with mice genetically deprived of metallothioneins due to the loss of functional MT I and II genes (coding for the 2 main isoforms of metallothionein involved in detoxification process) confirm the protective role of these proteins against cellular damages from metals such as cadmium or inorganic mercury (Satoh *et al.*, 1997; Klaassen and Liu, 1998).

#### 4.2.2. Cadmium spherocrystals

Cadmium-containing granules have been observed in the kidney of two white-sided dolphins (Gallien *et al.*, 2001). These two individuals with high Cd concentrations exhibited electron dense mineral concretions of diameters up to 300 nm in the basal membranes of the proximal tubule. These spherocrystals are made up numerous strata mineral deposits of calcium, phosphorus and cadmium. Cadmium has been detected with a molar ratio of Ca:Cd of 10:1 in the middle of these concretions.

The occurrence of metal-containing granules is well documented in invertebrates (Simkis, 1976) but this is the first report of granules containing cadmium in wild vertebrates. In these marine mammals exhibiting high levels of cadmium, these granules could constitute a way of immobilisation and detoxication.

## 5. LIMITS TO DETOXIFICATION AND CONCLUSIONS

Remarkable tolerance of marine mammals to heavy metals has been suggested through several detoxification processes as tiemannite storage and binding to metallothioneins, but is there a limit to the detoxification process and if so, what is the actual hazard of heavy metals?

The ratios between different metals appear more important than their absolute concentrations (Martin *et al.*, 1976; Becker *et al.*, 1995). Pups are more affected by these metals as they exhibit a higher methylmercury ratio compared to total mercury due to their poorly efficient detoxification mechanism (Wagemann *et al.*, 1988).

Moreover, a depressed molar ratio of Br:Hg:Se in premature pups of California sea lions was suggested to be a main death factor (Martin *et al.*, 1976).

Caurant *et al.* (1996) have proposed that detoxification of mercury could be limited in lactating longfinned pilot whales. Indeed, compared to other females, mercury concentrations were much higher in lactating females, while selenium concentrations were lower. Squid is the major food item of pilot whales, but a greater quantity and variety of fish species have been observed in the diet of lactating females. The authors suggested that the energy value of fish is higher than squid and a higher consumption of fish would cover the increasing need of energy to produce milk. The percentage of selenites (inorganic form of selenium) and the inorganic forms of mercury seem to be higher in squids than in fish muscles where methylated mercury is dominant. Most of selenium found in fish occurs in an organic form, which is less efficient than selenites in preventing the toxicity of mercury. The different diet of lactating females could induce both higher levels of mercury and a lower efficiency of the detoxification process (Caurant *et al.*, 1996).

Binding of cadmium to metallothioneins could also be a limiting factor to detoxification (Bouqueneau *et al.*, 1997). In debilitated sperm whales stranded on the Belgian coast, only a small amount of cadmium was bound to metallothioneins (Bouqueneau *et al.*, 1997; Holsbeek *et al.*, 1999). As quoted above, this could explain the observed debilitation which could have favoured their stranding. Precipitation of cadmium under a granule form could also lead to some toxic effect (Gallien *et al.*, 2001). The authors underlined the fact that lesions could be associated to these granules especially in older animals exhibiting high Cd concentrations and so, detoxification processes could lead to some toxic effects.

We have to keep in mind that any detoxification process has a cost for the cell or the organism involved and might have a threshold. This threshold cannot be fully defined in terms of tissue concentration because of the number of parameters that can interact to limit physiological pathways that lead to detoxification. For example, gender and hormonal activity can modulate the synthesis of metallothioneins (Blazka and Shaikh, 1991). Moreover, detoxification processes can lead to the formation of compounds which could have toxic effects. The accumulation and degradation of cadmium-metallothionein complex (CdMT) in the renal tubular epithelial cells can induce nephrotoxicity in mice counteracted by zinc which has a protective effect against this CdMT-induced nephrotoxicity (Liu *et al.*, 1996; Tang *et al.*, 1998). As a result of their physiological function in the homeostasis of essential metals, metallothioneins could be involved in many cellular pathways. Thus, they could modulate physiological process as an indirect effect of heavy metal exposure. For example, metallothioneins have been demonstrated as potential modulators of some parameters of the immune response (Leibbrandt *et al.*, 1994; Borghesi *et al.*, 1996). Detoxification pathways could therefore lead to more subtle toxic effects underlying the complexity of the toxic effects of heavy metals.

To conclude, the actual toxic effects of heavy metals on marine mammals remain unclear. Are they responsible - even in part - for the decline of some marine mammal species? As quoted above, that decline is obviously multifactorial: past overfishing, present increasing human activities, accumulation of pollutants among which heavy metals cannot be neglected. The role of marine mammals on the whole marine ecosystem is still poorly understood: their contribution in the recycling of nutrients is not very important, but their part in structuring marine communities and in modifying benthic habitats is more and more evident (Bowen, 1997). Marine mammals presently consume at least three times greater quantity of prey than do human fisheries, but that could be low compared to their ecological role, still poorly understood. Some species compete with fisheries, while others obviously do not, and yet others partly compete but could be useful to fisheries by regulating the development of non-commercial species, thereby limiting excessive competition with commercial ones (Bouqueneau *et al.*, 1997a). It is our belief that marine mammals deserve their place in the oceans and are worth being protected.

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