TOXICOLOGICAL INVESTIGATIONS ON THE HEAVY METALS CONTAMINATION OF SEABIRDS AND MARINE MAMMALS BEACHED ALONG THE BELGIAN COAST

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Seabirds and marine mammals are important top predators in the 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 lead to an important mortality, an increase of the frequency of strandings and a long term decline of the populations. In this framework, the strandings of birds and mammals along the Belgian coast are now registered and information on the status of the stranded animals is used to evaluate, following a multidisciplinary approach, the causes of mortality of the animals: the Pathology department of the Veterinary College of Liège University (Professor F.Coignoul) has in charge the necropsies and the histopathological evaluations, the Laboratory of Ecotoxicology and Polar Biology of the Free University of Brussels (V.U.B., Professor C.Joiris) the analysis of the mercury and organic xenobiotics contents, our team the heavy metals contents and their speciation. Our work is moreover made possible through the help of several rehabilitation centers at Oostende, Blankenberge, Nieuwpoort and Zwin, the collaboration of the Belgian Royal Institute of Natural Sciences (J.Tavernier) and the financial support of the Marine Sciences SSTC impulse programme.

The use of seabirds and marine mammals as bioindicators of the marine pollution and the estimation of the actual toxicity of the pollutant content of their tissues is not evident, and the responses of different species lead to different conclusions (*e.g.* seabirds collected in the North sea reveal high levels of copper while porpoises reveal high levels of cadmium, but both display high levels of mercury (Antoine *et al*, 1992)). Some species are able to detoxify heavy metals by binding to metalloproteins such as metallothioneins (Osborn (1978), Tohyama *et al* (1986), Bouquegneau *et al* (1996)) or by sequestration in various granules (see *e.g.* Bouquegneau and Joiris, 1988), both detoxifying mechanisms leading to high but non toxic concentrations in the tissues. A first step in an ecotoxicological approach is therefore the determination of the detoxication mechanisms. Finally, synergistic and/or antagonistic effects may occur between pollutants. For example, it is well known that zinc decreases the toxic effects of cadmium (*e.g.* Jonnalagadda and Prasada Rao, 1993).

Keywords: marine mammals - seabirds - heavy metals pollution - strandings

The actual toxicity of heavy metals, PCBs and other organic xenobiotics has been debated for a long time, but is obvious, even if it is often quite difficult, except in the case of oiling for seabirds, to relate the mortality of birds and mammals to one single pollutant. The cause of the debilitation and/or of the death is generally multifactorial. This has been recently shown by Swart *et al* (1994) which have demonstrated that impaired immunological functions in harbour seals were associated with chronic exposure to environmental contaminants accumulated through the marine food web.

Common guillemots (*Uria aalge)* are, by far, the most numerous pelagic species stranding on the Belgian coast and have been particularly studied in our laboratory. Like other seabird species stranding in the same area, they contain particularly high levels of copper (Bouquegneau *et al*, 1994 & 1996). Zinc and cadmium hepatic contents of these guillemots are on the contrary within the range reported in other seabird species. A direct comparison with data about common guillemots collected in the northwest Scotland (Stewart *et al*, 1994) and in the northern Norway (Wenzel and Gabrielsen, 1995) clearly shows that those which were stranded on the Belgian coast (Bouquegneau *et al*, 1994) are heavily polluted by copper, zinc and mercury. The mortality of non-oiled guillemots *Uria aalge* stranded along the Belgian coast has been attributed to the development of hemorrhagic gastroenteritis which is related, via cachexia, to high levels of copper, zinc and cadmium (Bouquegneau *et al*, 1994). In man, it is known that ingested copper salts are able to induce gastroenteritis and that zinc salts are irritative for the digestive tract (Lauwerys, 1990). Cadmium has been shown to induce cachexia and entheropathy in the Japanese quail (Fox and Fry, 1970; Richardson and Fox, 1974). As the most important part of their excess was not found to be bound to metallothioneins (Bouquegneau *et al*, 1996), we think that copper, zinc and cadmium have a harmful effect on the guillemots stranded along the Belgian coast.

Whilst the strandings of seabirds are quite numerous, the beaching of marine mammals is much more uncommon, so that it is difficult to make general conclusions about their contamination levels. However, the recent stranding of four debilitated sperm whales *Physeter macrocephalus* at Koksijde and Nieuwpoort (November 18th, 1994) allowed a somewhat more precise investigation (Joiris *et al, in preparation*).

Like other aquatic organisms, marine mammals are able to bind metals such as zinc, cadmium, copper and inorganic mercury to metallothioneins (see *e.g.* Tohyama *et al* (1986)). On another hand, Koeman *et al* (1973) first reported a causal relationship between mercury and selenium in marine mammals. After that, Martoja and Berry (1980) have identified particles of pure tiemannite (HgSe) stored in the lysosomes of the connective tissue of the liver of goose-beaked whales *Ziphius cavirostris*. Since these works, many authors have confirmed both the correlation between selenium and mercury, and the presence of tiemannite in the lysosomes of liver and kidneys of marine mammals (see *e.g.* Nigro and Leonzio, 1993). Both detoxifying mechanisms leading to high but non toxic concentrations in the tissues and the speciation of the metal in the tissues of marine mammals are here again needed to evaluate its potentially toxic concentration. Few data are available in the literature which would allow 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 (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 cadmium levels appeared to be quite high. The mercury content of the muscles (Joiris *et al, in preparation*) was 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). No detectable amount of inorganic mercury was found to be bound to metallothioneins, but a close relationship was shown between selenium and mercury contents of the liver, which suggests a detoxification of methylmercury under the tiemannite form. On the contrary, the cadmium content of the liver was very high, twice the one of the livers of the North Pacific sperm whales described by Ridlington *et al* (1981), but however in the range of the liver cadmium concentration of mammal species which are feeding on cephalopods (Thompson, 1990). Such a large level of cadmium was also found in other sperm whales stranded around the North sea during the same period (Law *et al*, 1996). This suggests a potential toxicity of cadmium which is enhanced by the study of the speciation of the metal: only a small part of the cadmium appears to be detoxified through binding to metallothioneins compared with the livers of sperm whales stranded in the North Pacific (Ridlington *et al*, 1981). This suggests that cadmium, on the contrary with zinc and copper which are normally bound to metallothioneins, is potentially highly toxic for the animals. This is at variance with the concept that marine mammals have had to develop adaptations against naturally occuring inorganic contaminants like cadmium. In fact, natural cadmium pollution is associated with zinc pollution, since cadmium is a metallic element which is naturally present in soil and rocks in association with zinc. Zinc decreases the toxicity of cadmium, but, in this case, the zinc content of the sperm whales was lower than that found in those from the Pacific and described by Ridlington *et al* (1981). Cadmium, which was not, as it is usually the case, detoxified either by metallothioneins or by high zinc content, is therefore to be considered as a potential factor responsible for the debilitation of the animals. Further research is obviously needed to definitively conclude about the actual causes of the strandings of these animals.

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