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## Bone Fluoride Concentrations in Beluga Whales from Canada

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**ABSTRACT:** Beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary have been reported to have dental and bone abnormalities. To determine whether these lesions could be caused by high exposure to fluorides, we measured bone fluoride levels in eight beluga whales stranded on the shores of the St. Lawrence Estuary (Quebec, Canada), and in nine beluga whales killed by Inuit hunters in the Hudson Bay (North Western Territories, Canada). In both groups, fluoride concentrations were higher than those found in terrestrial mammals intoxicated by fluorides. Unexpectedly, fluoride concentration was significantly higher in beluga whales from the Hudson Bay ( $\bar{x} \pm \text{SD}$ : 10,365  $\pm$  1,098 ppm) than in beluga whales from the St. Lawrence Estuary (4,539  $\pm$  875 ppm) and was positively correlated with age in the latter population. Differences in diet might explain the differences in fluoride concentrations found between these two populations.

**Key words:** Beluga whale, bone, *Delphinapterus leucas*, fluoride, toxicosis.

An indigenous population of approximately 500 beluga whales (*Delphinapterus leucas*) inhabits the St. Lawrence Estuary at the mouth of the Saguenay Fjord (48°07'N, 69°43'W; Quebec, Canada). This population is chronically exposed to a complex mixture of industrial pollutants (De Guise et al., 1994; Martineau et al., 1994). High levels of fluorides are naturally present in some environments, including seawater, and can result in chronic fluorine toxicosis in wildlife (Shupe et al., 1984). Fluorides also are widely used in a variety of industries, such as aluminium smelters (Krook and Maylin, 1978) and paper mills (National Toxicology Program, 1990; Paranjpe et al., 1994). Fluoride

emissions from aluminium plants (Krook and Maylin, 1978; Suttie et al., 1987; Vikøren and Stuve, 1995, 1996) and from petrochemical sites (Paranjpe et al., 1994) have resulted in chronic fluorine toxicosis in domestic and wild terrestrial mammals.

Fluorine toxicosis has been reported in a variety of domestic and wild terrestrial mammals, including cattle, wild cervids and rodents (Krook and Maylin, 1978; Paranjpe et al., 1994; Shupe et al., 1984; Suttie et al., 1987; Vikøren and Stuve, 1996) but not in marine mammals. Major targets in chronic fluorine toxicosis are teeth and bones. Dental lesions develop if intoxication occurs while teeth are in the developmental stage and begin by small dry and chalky foci in the enamel (mottling) (Shupe et al., 1963). When the change is more severe, all the enamel can be affected, predisposing the teeth to excessive decay. Osteofluorosis (bone fluorosis) can be observed at any age and results in the development of exostoses, giving a chalky roughened appearance to the affected bones.

Beluga whales from the St. Lawrence Estuary and from the Saguenay Fjord have been reported to have high prevalence of overworn teeth and occasionally vertebral exostoses (Martineau et al., 1988; De Guise et al., 1995). Because sources of industrial fluorides (aluminium smelters and paper mills) are located on the shores of the Saguenay River, fluorine toxicosis was included in the possible causes of these dental and osseous lesions. Thus, we measured

bone fluoride levels in beluga whales from the St. Lawrence Estuary, and for a control group we used beluga whales from the Hudson Bay, a relatively unpolluted area of the Canadian Arctic.

Eight whales from the St. Lawrence population were found dead, stranded on the shore in 1994 and 1995. Death was attributed to severe verminous bronchopneumonia caused by *Halocercus monoceris* in three animals (DL-07-95, DL-09-95 and DL-12-95), a dissecting aneurysm of the pulmonary trunk (DL-11-95), and an abdominal laceration caused by a motor boat propeller (DL-05-95). One animal was an orphaned calf (DL-08-95) and the cause of death was not found in two cases (DL-03-95 and DL-06-95). Nine beluga whales from the Eastern Hudson Bay were killed for food consumption by Inuit hunters in August 1995 at Arviat (61°12'N, 94°01'W; North Western Territories, Canada). Two teeth from an adult (>31-yr old) beluga whale of the St. Lawrence Estuary (DL-03-95) showed a large 1 cm wide ring of mottled enamel at the base of the crown (Fig. 1). All other teeth ( $n = 18$ ) from this animal also were affected, although to a lesser extent. Enamel changes or exostoses were not observed in the other belugas from the St. Lawrence Estuary and from the Hudson Bay.

A 5 cm long cross-section from the middle of the last left rib was sampled from each carcass. This bone was selected because it is easy to remove in field conditions and because information on fluorine levels for this specific bone were available in cattle (Shupe et al., 1963; Krook and Maylin, 1978). The bone was cleaned by boiling for 1 hr, and any adhering tissue was further removed with a razor blade. Fluoride concentration was estimated with the use of a fluoride electrode using a method previously described (Paranjpe et al., 1994). Briefly, the bones were placed in petroleum ether for 72 hr and ether was changed twice daily. The bones were then dried overnight in an oven, ashed at 550 C, weighed, and then pulverized into a

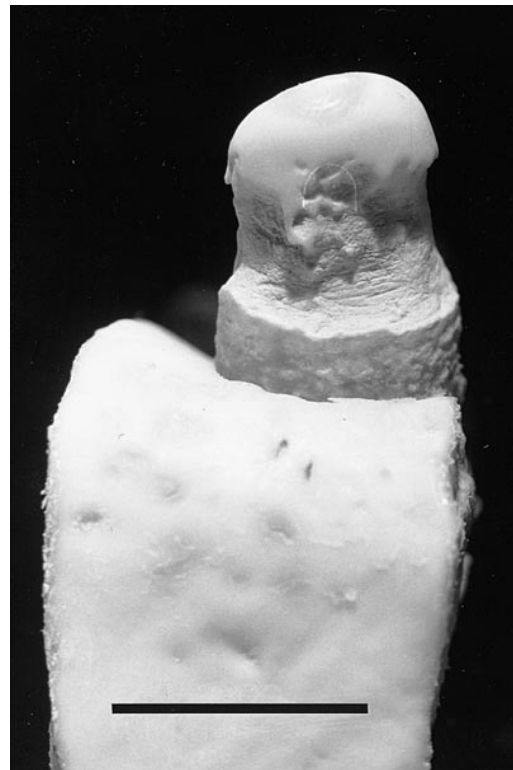


FIGURE 1. Annular mottling of the enamel of the third tooth of the maxilla from beluga whale DL-03-95. The bar represents 1.5 cm.

fine powder. The ash was dissolved in 2 ml of 0.25 M HCl, to which 8 ml of TISAB IV buffer was added (prepared using the formula provided by the manufacturer of the ionalyzer: Orion Research Incorporated, Boston, Massachusetts, USA). Bone fluoride concentration was determined with an Orion Research Digital Ionalyzer Model 701A equipped with a fluoride-specific electrode (Orion Research Incorporated) and expressed in parts per million. Aging was carried out by counting dentine growth layers on longitudinal sections of teeth, adopting the standard of two growth layer groups per year (Sergeant, 1973).

The relationship between the bone fluoride concentration and beluga age was tested using Spearman rank correlation. A logistic regression, using the age as continuous independent variable of interest, was calculated to measure the statistical signif-

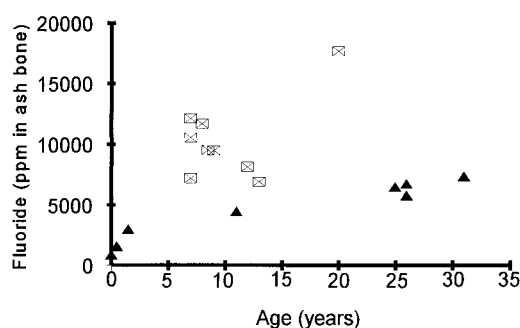


FIGURE 2. Relationship of age (years) and rib fluorine (F) content (ppm F in bone ash) for beluga whales sampled in Hudson Bay (□) and in the St. Lawrence Estuary (▲).

ificance of the age as a factor in the fluoride concentration of bone in both populations. The level of significance was set at  $\alpha = 0.05$ . Statistical analyses were performed using the SAS 7.0 software (SAS Institute Inc., Cary, North Carolina, USA).

Bone fluoride levels increased with age in whales from the St. Lawrence Estuary (Fig. 2;  $r = 0.80$ ,  $P < 0.01$ ), but not in animals from the Arctic ( $P = 0.11$ ). Fluoride concentrations were significantly higher in beluga whales from the Arctic compared to beluga whales from the St. Lawrence Estuary ( $P = 0.01$ ; Table 1). The beluga whale from the St. Lawrence Estuary with enamel defects (DL-03-95) had a lower rib fluoride level than the level ( $\bar{x} \pm SD$ ) in whales from the Arctic ( $7,366 \pm 875$  ppm versus  $10,365 \pm 1,098$  ppm, respectively).

Bone fluoride levels apparently have not been previously assessed in Odontoceti. The fluoride levels that we report here are similar to, or even higher than those of fluorotic domestic (Shupe et al., 1963; Krook and Maylin, 1978) and wild (Shupe et al., 1984; Paranjpe et al., 1994; Vikøren et al., 1996) terrestrial mammals, and wild birds living in areas exposed to fluoride emissions (Vikøren and Stuve, 1995). We hypothesize that these high levels were not clinically significant because lesions compatible with fluorosis were not found in beluga whales from the Arctic and because

TABLE 1. Rib fluoride concentration in beluga whales from the Hudson Bay and from the St. Lawrence Estuary.

	Sex <sup>b</sup>	Age <sup>c</sup>	Bone fluorine <sup>d</sup>
Hudson Bay			
A-05	F	7.0	10,544
A-10	M	7.0	7,186
A-07	M	7.0	12,126
A-01	M	8.0	11,670
A-02	M	8.5	9,519
A-04	M	9.0	9,491
A-08	M	12.0	8,148
A-06	M	13.0	6,923
A-13	F	20.0	17,686
Mean $\pm$ SD <sup>a</sup>		10.2 $\pm$ 4.3	10,365 $\pm$ 1,098
St. Lawrence			
DL-08-95	F	0	873
DL-07-95	M	0.5	1,581
DL-09-95	M	1.5	2,996
DL-12-95	M	11.0	4,461
DL-06-95	M	25.0	6,508
DL-11-95	M	26.0	5,789
DL-05-95	F	26.0	6,742
DL-03-95	M	31.0	7,336
Mean $\pm$ SD <sup>a</sup>		15.1 $\pm$ 13.3	4,539 $\pm$ 875

<sup>a</sup> Standard deviation.

<sup>b</sup> M (male), F (female).

<sup>c</sup> Years.

<sup>d</sup> ppm on the basis of bone ash weight.

the only beluga whale in this series, having dental lesions suggestive of fluorosis (DL-03-95), had bone fluoride levels lower than the apparently healthy whales from the Arctic. Similarly, bone fluorine levels are elevated in Mysticeti whales, with ( $\bar{x} \pm SD$ ) levels of  $9,920 \pm 4,100$  ppm in fin whales, *Balaenoptera physalus* (Landy et al., 1991), and  $6,510 \pm 2,969$  ppm in minke whales, *Balaenoptera acutorostrata* (Alne 1995), and are not associated with clinical signs of fluorosis.

Other causes of enamel hypoplasia in domestic mammals include infection by distemper virus and bovine virus diarrhea virus (Barker et al., 1993). However, beluga whales from the St. Lawrence Estuary are seronegative to these viruses (Mikaelian et al., 1999).

Food and drinking water are the major sources of fluorides in domestic (Krook

and Maylin, 1978) and wild mammals (Shupe et al., 1984; Vikøren and Stuve, 1996). Among the dietary sources of fluorides, whole fish and krill are the most important (Soevik and Braekkan, 1981; Rao et al., 1995), and fluorides concentration markedly differs among fish species (Stewart et al., 1974). Beluga whales from the St. Lawrence Estuary (Vladykov, 1946) and from the Arctic Ocean (Lowry et al., 1985) mostly feed on fish and invertebrates, including shrimp (Braham et al., 1984). This diet presumably results in high fluoride intake. We hypothesize that the difference in bone fluoride concentration between beluga whales from the Arctic and from the St. Lawrence Estuary could reflect a difference in the diets of these two beluga whale populations.

The positive correlation between fluoride levels and age in beluga whales from the St. Lawrence Estuary suggests a life-long accumulation, a finding that also has been observed in Mysticeti (Alne 1995; Landy et al., 1991), as well as in domestic (Krook and Maylin, 1978) and wild (Kierdorf et al., 1995) ungulates. The apparent absence of a similar increase with age in beluga whales from the Arctic is probably due to the small age range of this sample, which did not allow to test satisfactorily the relationship between bone fluoride concentration and age.

These results strongly suggest that dental and bone lesions occasionally observed in beluga whales from the St. Lawrence Estuary are not caused by high exposure to fluorides and that beluga whales, as a species, are probably resistant to high fluorine intake. Further studies are required to ascertain the causes of higher bone fluoride levels in beluga whales from the Arctic.

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