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Metal Concentrations in the Vertebrae of the Dogfish, *Centroscymnus crepidater* (Bocage and Capello) and *Deania calcea* (Lowe)

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A number of studies have determined metal concentrations in elasmobranch muscle and livers (Turoczy et al, 2000), but the bioaccumulation and bioconcentration of metals in shark vetebrae is not well understood. This is especially true for elasmobranchs in Australian waters. In this study, we investigated the concentrations of metals in the cartilagenous vertebrae of the golden (or long-nosed) velvet dogfish, *Centroscymnus crepidater*, and the brier (or shovelnose) shark, *Deania calcea*. These dogfish were captured at depths of approximately 600 m in the Southern Ocean south of Victoria, Australia. Deep sea dogfish, such as *C. crepidater* and *D. calcea*, contribute to the trawl component of the Australian South East Fishery. Since they are now legally sold for human consumption, metal accumulating in the vertebrae of these long lived but little studied shark may pose a human health threat if the vertebrae are incorporated into pharmaceutical products, as occurs in South Australia. Herein we report the results of our survey, and where relevant compare our results to the maximum values permitted by the Australian Food Standards Code and the human health implications for consumers of this material discussed.

MATERIALS AND METHODS

The dogfish were captured off the coast of Victoria by a commercial otter board trawler during April and May 1998, and were frozen until processing. They were then thawed and identified to species level prior to dissection. The vertebrae of two *D. calcea* (1 male, 1 female), and six *C. crepidator* (4 male, 2 female) were dissected and cleaned in the following manner : samples of dogfish vertebrae were dissected from immediately underneath the leading edge of the first dorsal fin, since vertebrae in this region are usually the largest. Remnant tissue was softened by immersing the sample in a very hot water bath for 2-3 minutes, then removed with forceps and scalpels. The samples were then freeze dried, and stored frozen until analysis. At that time, the samples were ground to a fine powder using a marble pestle and mortar.

Deionised water having a resistivity of at least 18 MΩ cm^{-1} was produced by

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passing singly distilled water through a Milli-Q Water Purification System. Ultrapure analytical reagent grade nitric acid (68% w/v HNO_3) and perchloric acid (70% w/v $HClO_4$) were obtained from Tama Chemicals Co. Ltd, Kawasaki City, Japan.

For metals other than mercury, vertebrae were digested using a mixture of nitric acid / perchloric acid. The digests were filtered through 0.2 μm cellulose acetate disposable syringe filters (Sartorius CE Minisart RC15, Sartorius, Germany) prior to analysis. Digests were analysed using an ICP-61E (Thermo Jarrell Ash, Japan). The following analytical wavelengths were monitored : Al, 167.081 nm; As, 189.042 nm; Ca, 317.933 nm; Cd, 214.438 nm; Co, 238.892 nm; Cr, 267.716 nm; Cu, 324.754 nm; Fe, 259.940 nm; K, 766.400 nm; Mg, 285.210 nm; Mn, 257.610 nm; Na, 330.232 nm; Ni, 231.604 nm; Pb, 220.353 nm; Sr, 421.552 nm; Zn, 206.200 nm. Mercury concentrations were determined after vaporisation of powdered vertebrae in the vaporisation chamber of a Mercury Atomiser MA-1S (Nippon Instruments, Japan) connnected to an external Mercury Detector MD-1 (Nippon Instruments, Japan). Reference material (NIES CRM No. 6, Mussel), obtained from the National Institute for Environmental Studies, Japan, was used to assess the performance of the analytical methods. Reagent blanks were also processed, with nitric acid quantities adjusted to maintain similar treatment volumes in samples and blanks.

RESULTS AND DISCUSSION

Analysis of the certified reference material (NIES CRM No. 6, Mussel, National Institute for Environmental Studies, Japan) found all certified and reference elements (except Ag, Al, Cd, Co, Cr, Ni and Pb) to be within 16% of expected values (90-116% recovery; Table 1). Silver was not determined, Al recovery was poor (~50% of reference value), while the concentrations of Cd, Co, Cr, Ni, and Pb were below the detection limits of the instrumental techniques employed. Based on the analysis of NIES CRM No. 6, quantitative results were expected for eleven elements (As, Ca, Cu, Fe, Hg, K, Mg, Mn, Na, Sr and Zn), all of which were detected in the dogfish vertebrae. Only these elements are considered hereafter. Metal concentrations in the shark vertebrae are summarised in Table 2. Values quoted have not been corrected for analyte recoveries from certified reference materials.

The vertebrae analysed in this study was found to comply with the Australian Food Standards Code maximum concentrations for copper, lead, mercury and zinc of less than 10.0, 1.0, 1.0 and 150 mg kg^{-1} wet weight, respectively (Australia New Zealand Food Authority, 1998; Divide values quoted in Table 2 by 5 to obtain approximate wet weight concentrations).

Table 1. Summary of NIES CRM No. 6, Mussel certified and reference (*) metal concentrations, and concentrations determined in this study.

	NIES CRM No. 6, Mussel				
Element	Certified value	Observed value	Element	Certified value	Observed value
	(mg kg^{-1})			(mg kg^{-1})	
Ag	0.027 – 0.003	ND	Ni	0.93 – 0.06	< 0.02
Al	220 *	107 – 10.7	Pb	0.91 – 0.04	< 0.05
As	9.2 – 0.5	10.67 – 1.5	Se	1.5 *	16.69 – 0.6
Cd	0.82 – 0.03	< 0.01	Zn	106 – 6	107 – 2.1
Co	0.37 *	< 0.01			
Cr	0.63 – 0.07	< 0.01		(%)	
Cu	4.9 – 0.3	4.43 – 0.2	Ca	0.13 – 0.01	0.13 – 0.01
Fe	158 – 8	141 – 1.1	K	0.54 – 0.02	0.52 – 0.01
Hg	0.05 *	0.05 – 0.01	Mg	0.21 – 0.01	0.20 – 0.00
Mn	16.3 – 1.2	15.57 – 0.2	Na	1.00 – 0.03	0.95 – 0.01

Values quoted on a dry weight basis. ND, not determined. The minimum determinable limits (MDL) indicated are based on mean – 10 standard deviations for the digested blanks (American Public Health Association, 1995).

Table 2. Metal concentrations in vertebrae (this study) and muscle (Turoczy et al, 2000) of *D. calcea* and *C. crepidator* determined by ICP-AES after acid digestion of samples.

	Vertebrae metal concentration				
Metal	*D. calcea* (n = 2) *	*C. crepidator* (n = 4) **	Metal	*D. calcea* (n = 2) *	*C. crepidator* (n = 4) **
	(mg kg^{-1})			(mg kg^{-1})	
As	27.9 ± 5.2	19.50 ± 3.2	Zn	67.5 ± 18.5	34.1 ± 4.9
Cu	< 0.01	1.0 ± 1.2		(%)	
Fe	13.6 ± 2.1	7.6 ± 1.6	Ca	11.4 ± 3.4	13.8 ± 2.0
Hg	0.46 ± 0.37	0.24 ± 0.2	K	0.8 ± 0.4	0.5 ± 0.2
Mn	17.7 ± 7.2	20.2 ± 1.5	Mg	0.2 ± 0.0	0.2 ± 0.0
Sr	598 ± 162	715 ± 112	Na	0.9 ± 0.1	1.0 ± 0.2

Values quoted on a dry weight basis; *, range quoted represents half the distance between two values ; ** , range quoted is one standrard deviation from the mean.

The Australian Food Standards Code specifies that the maximum concentration of inorganic arsenic permitted in fish is 1.0 mg kg^{-1} wet weight (Australia New Zealand Food Authority, 1998). The concentration of As in the vertebrae of both species exceeded this limit. However, the concentration of As in the vertebrae was such that consumption of approximately 50 or 35 g per week of *C. crepidater* and *D. calcea* vertebrae, respectively, would be required to exceed the United Nations Food and Agriculture Organisation s Provisional Weekly Tolerable Intake (PWTI) of 1050 µg As for a 70 kg body weight person (R. Ellis, Acting Secretariat to Joint FAO/WHO Expert Committee on Food Additives, personal communication, 2000). Preliminary studies using laser ablation inductively coupled plasma mass

spectrometry (LA-ICP-MS), suggest that As, and Hg, are concentrated in the pulp cavity of the vertebrae, probably in the remains of the spinal cord. Thus, removing the spinal cord before drying will reduce As concentrations in vertebrae significantly.

Arsenic found in marine organisms is found in the form of fat or water soluble organoarsenic compounds, such as arsenobetaine (IPCS, 1981), and these are considered to be several orders of magnitude less toxic than inorganic arsenic. In addition, seafood As been found to be readily absorbed by the human gastro-intestinal tract. Fortunately, it is also rapidly excreted, and there appears to be no conclusive evidence for long-term toxicity or carcinogenic effects from ingestion of seafood arsenic. Thus, even excessive consumption of *C. crepidater* or *D. calcea* vertebrae is unlikely to cause harm from ingestion of the arsenic the vertebrae contain.

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