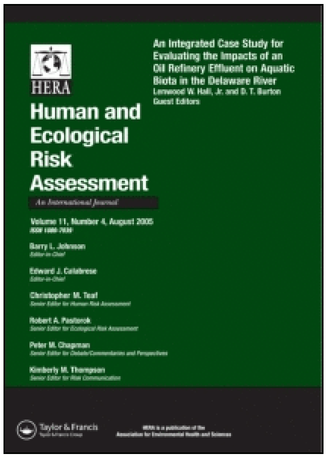

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Trace Metals (Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn) in Food Supplements of Marine Origin

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Trace Metals (Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn) in Food Supplements of Marine Origin

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ABSTRACT

We determined the concentrations of Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn in dietary supplements of marine origin. Four supplement categories were studied; algae, coral, krill, and shark cartilage. A direct mercury analyzer was used for Hg determinations while acid digestions and ICP-AES were used for Cr analysis and ICP-MS for the other trace metals. Algae are the supplements showing the highest concentrations of Pb, Cr, and Ni with respective means of 1.6 mg Pb/kg dry weight (d.w.), 3.2 Cr mg/kg d.w., and 8.0 mg Ni/kg d.w. Krill supplements have the highest levels of Cd, Cu, and Zn with 0.65 mg Cd/kg d.w., 63 mg Cu/kg d.w., and 50 mg Zn/kg d.w., respectively. Shark cartilage supplements show the highest levels of Hg and Co with mean concentrations of 160 μg Hg/kg d.w. and 73 ± 51 μg Co/kg d.w., respectively. No samples in our study exceeded the provisional tolerable daily intakes set by Health Canada, the joint committee of the World Health Organization/Food and Agricultural Organization, or the U.S. Environmental Protection Agency. Nevertheless, Ni and Pb in algae and Hg in shark cartilage may end up contributing to a very significant portion of the allowable daily intake—leaving little room for normal intake through food consumption and other exposure pathways.

Key Words: algae, coral, krill, shark cartilage, trace metals, nutraceuticals, natural health products, dietary supplements.

INTRODUCTION

Dietary supplements are now in common use in the population. It is estimated that from 50 to 71% of the North American population regularly consumes dietary supplements (Nestmann *et al.* 2006). This trend can be attributed to the health benefits attributed to the consumption of the different supplements (Grollman 2005). Dietary supplements can also be named in various ways, food supplements, nutraceuticals, functional foods, natural health products, and so on. The key aspect to consider is that whatever the name used, they will usually be subjected to hybrid

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regulations falling in between foods and drugs. Depending on the country (Brownie 2005; Grollman 2005; Gulati and Ottaway 2006; Nestmann *et al.* 2006), the exact obligations (if any) of the producers and distributors vary enormously and in most cases, the regulatory verifications and quality control of the product safety remains minimal. In most legislation, the objective is to ensure that the manufacturers offer a product free of danger to the consumers at the recommended directions for use. In addition, manufacturers usually have the obligation to indicate the appropriate warnings associated with the use of their product. But, even with these regulations, producers are not submitted to minimal manufacturing standards for the sale and quality control of dietary supplements, thus implying larger distribution potency and larger availability of natural products for the general public.

In Canada, dietary supplements are submitted to the Canadian Food Inspection Agency (CFIA) supervision. A new regulation aiming at the control of natural health products in Canada stipulates that, for every new natural product getting on the market, the health benefits being displayed on the label must be validated with appropriate scientific proofs (Nestmann *et al.* 2006). Nevertheless, manufacturers of natural products that were already distributed before the onset of the new regulation have until year 2010 to conform to the new rules, implying that many dietary supplements on the market at this time will suffer from a lack of control for some years to come.

The bioaccumulation properties of algae, coral, krill, and shark can potentially lead to high concentrations of potentially health-damaging contaminants. More specifically, lead concentrations above the provisional total tolerable intake (PTTI) set by the International Program of Chemical Safety of the World Health Organisation (IPCS/WHO) for children and pregnant women were measured in spirulina and shark cartilage supplements (Kay 1991).

Our objective was not to evaluate if the promoted health benefits are real and tangible; we simply wanted to measure the concentration of trace elements within those supplements and evaluate whether taking the proposed dose of the supplements could lead to potential health risks. To that purpose, we have chosen to focus on four categories: algae supplements, coral, krill, and shark cartilage supplements.

Dietary supplements of marine origin are experiencing a large increase in consumption, presumably as a result of the perceived health benefits. The high content of various nutritive elements in algae is well known and algae can be an important nutritional source of minerals and proteins (Rupérez 2002). In fact, micro-algae are already used in different spheres of activities to provide proteins, vitamins, soluble lipid compounds, glucolipids, and sulfolipids sources (Kay 1991) or for the treatment of iodine deficiencies (Singh *et al.* 2005).

Coral supplements were included in our range of dietary supplements because they may be an important and a presumably interesting source of organic, “bioavailable” calcium for the prevention of osteoporosis or to supplement calcium in the diet. Krill supplements were included because they are rich in fatty acids, including the essential omega-3 polyunsaturated fatty acids, and in antioxidants compounds. Antarctic krill, for example, contains large quantities of eicosapentanoate and docosahexanoate along with antioxidants of the carotenoids family like the astaxanthin and its mono- and di-esters found principally in the carapace of crustaceans (Takaichi *et al.* 2003). This category of supplements may represent a good source of essential polyunsaturated fatty acids for those who do not eat fish products on a regular basis.

Shark cartilage is also a dietary supplement sold with presumed high healing capacities for diseases like cancer, arthritis, and osteoarthritis. Shark cartilage contains two angiogenesis inhibitors, U-995, a molecule composed of two small proteins, and SCF2, a proteoglycan compound (Sheu *et al.* 1998). Shark cartilage compounds have shown promising *in vitro* results for fighting cancer in some assays (Feyzi *et al.* 2003; Hagedorn and Bikfalvi 2000; Hassan *et al.* 2005). But, the benefits of consuming shark cartilage capsules on tumor development have not yet been demonstrated in clinical studies and doubts remain (Ritter 2004).

In our experiment, we studied mercury, cadmium, and lead, three trace metals often suspected of causing human health concerns. The other five metals that are part of this study are chromium, cobalt, copper, nickel, and zinc, and they are considered essential elements in human nutrition, but these metals can also become potentially toxic if consumed in excessive amounts.

MATERIALS AND METHODS

Contamination Control

All glassware and plasticware used in this experiment were washed manually with hot water and a brush using a phosphate-free laboratory detergent. They were rinsed three or more times with warm tap water, followed by three or more rinses with distilled water, and then an overnight soaking in a 10% HNO_3 acid bath. We then rinsed three or more times with distilled-deionized water (dd-H_2O-Milli-Q-RG, Millipore Ultra Pure Water System) with 18 mΩ.cm conductance.

Samples

The 55 samples of marine food supplements used in this experiment were bought in 2004 and 2005 in drugstores, supermarkets, and natural food stores in the Montreal area (QC, Canada), on the Internet in a U.S. dietary supplements shop and in Australia for three samples of shark cartilages (Adelaide, NSW). We collected a total of 55 samples: 31 algae products (kelp, spirulina, chlorella, sea weed), 16 shark cartilages samples, 5 coral samples, and 3 krill supplements.

The list of supplements including the maximum daily use (MDU) suggested on the bottle for adults and children were recorded. Unless specific instructions were given for children, we assumed that the maximum daily use indicated on the bottle was applicable to all. All concentrations are reported on a dry weight basis.

Sample Preparation

For supplements presented in caplets, eight or more caplets were powdered by hand using an agate mortar and pestle (Cubadda and Raggi 2005). In the case of supplements in capsules, only the powder inside the capsules was used and considered for the capsule weight. In both cases, powder for each sample was homogenized, dried in a forced-air oven at 40°C, weighted, and transferred to a HDPE bottle for storage until needed. The mean weight of each capsule was recorded.

Reference Samples

A total of three reference samples were used in this study; two for the mercury determination and one for the other trace metals . Tort-2 is a reference material for trace elements made from lobster hepatopancreas, certified by the National Research Council of Canada (NRCC). SO-2 is a soil reference material certified by the Canada Centre for Mineral and Energy Technology (CCMET). IAEA-405 is an estuary sediment material furnished by the International Atomic Energy Agency, certified by the IAEA Marine Environment Laboratory.

Mercury

Total mercury concentrations were measured using a Direct Mercury Analyser 80 (DMA-80) (Milestone Inc, Monroe, CT, USA) coupled to a computer using the DMA-80 data acquisition program version 4.0.8.0. Details on the functioning and operation of the DMA-80 can be found elsewhere (Fayad *et al.* 2004). The method limit of detection (MLD) with this instrument was 0.1 ng of Hg. To obtain this value, sixteen blanks were analyzed (data not shown) in the DMA-80 and the MLD was calculated by multiplying the standard deviation of the blanks by a factor of three (3 × s).

Method validation was made by using Tort-2, SO-2, and IAEA-405 as reference materials. The reference material samples were analyzed every ten samples to ensure instrument performance. For the Tort-2, the certified mercury concentration from the NRCC is 0.27 ± 0.06 mg of Hg kg^{-1} and we measured 0.293 ± 0.002 mg of Hg kg^{-1}. The sandy soil reference sample, SO-2, was measured at 0.0801 ± 0.0001 mg of Hg kg^{-1} with the certified value of 0.082 mg ± 0.009 mg of Hg kg^{-1} given by the CCMET. Finally, the IAEA-405 reference sample had a 4.7% deviation between the certified (0.82 ± 0.04 mg of Hg kg^{-1} and the measured mercury concentrations (0.85 ± 0.08 mg of Hg kg^{-1}.

Cd, Co, Cr, Cu, Ni, Pb, and Zn

Sample digestion

Sample digestion was made using a DK 42/26 Heating Digester (Velp Scientifica, Milano, Italia) in which 42 26 × 330 mm digesting tubes can be placed simultaneously. About 0.500 g of supplement samples was weighed on an analytical balance (Sartorius Analytic) directly in the digesting tubes. Ten mL of nitric acid (Trace Metal grade, Fisher Scientific) were added to each tube using an adjustable volumetric dispenser. The tubes were then covered with Parafilm paper and left under a fume hood overnight to begin the digestion.

Before placing the tubes on the digester, Parafilm papers were replaced with watch glasses and the digestions were performed by heating the samples at 150°C for one hour. After one hour, the few samples that were not completely digested were reheated for one hour to complete the digestion. Method control and validation were made by randomly placing digestion blanks and reference samples every ten or twelve real samples. After digestion, samples were removed from the digester and kept under a fume hood until cool. Each sample solution was then filtered directly into a 100-mL volumetric flask using filter paper (Fisher Scientific Q5).

Solutions were then completed to volume with distilled-deionized water (dd-H_2O) and transferred into HDPE bottles for storage. All supplements were digested and analyzed in duplicate.

Analysis of trace metal concentrations

The concentration of the six trace metals (Cd, Co, Cu, Ni, Pb, Zn) was measured using a Varian UltraMass 700 ICP-MS. For ICP-MS analysis, digest sample solutions were diluted to a final concentration of 4% nitric acid: 20-mL of sample was transferred into a 50-mL volumetric flask and dd-H_2O added for volume completion. The concentrations of Cr were measured using an ICP-AES (Iris advantage/1000 from Jarrell Ash corporation, U.S.A) using the same digests.

The digestion analysis was validated using the Tort-2 reference material. The results for the trace metal concentrations obtained by ICP were compared to the certified values for the Tort-2. Concentration deviations between experimental and certified values are in the range of –11.3% to +18.2% for Cd, Co, Pb, Cu, Cr, Ni, and Zn.

RESULTS AND DISCUSSION

Trace Metal Concentrations in Supplements

The mean concentrations of trace metals in the different categories of marine food supplements are presented in Table 1. Each metal is discussed individually.

Provisional Tolerable Daily Intakes (PTDI)

To determine if the consumption of the food supplements could have a negative impact on human health, we have calculated the daily intake (DI) of the different trace metals if the given maximum daily direction of use was followed by the consumers. Calculations were made assuming a 70-kg of body weight for adults and a 13-kg body weight for children (Dolan *et al.* 2003);

$$\mathrm{DI} = \frac{[\text{Metal in caplet}] \times [\text{mass of caplet}] \times \text{max dose}}{\text{Body weight (13 or 70 kg)}} \tag{1}$$

The values were compared to the provisional tolerable daily intakes (PTDI) established by the joint committee of the World Health Organization and the Food and Agriculture Organization (WHO/FAO) (WHO 1989) or the reference dose (RfD) established by the U.S. Environmental Protection Agency (USEPA 2005). Percentages of the PTDI for each supplement (except Co) are displayed in Figure 1. Cobalt was not included in this calculation because we found no PTDI values.

Cadmium concentrations

Cadmium measured in the marine food supplements ranged from 0.016 mg/kg to 1.9 mg/kg (Table 1). There seems to be little or no large differences among the supplement categories except for one algae outlier value clearly higher than the other concentrations at 1.9 mg /kg (individual supplement data not shown). Those results do not differ from the values that have already been reported in the literature for cadmium in different food products where the overall range of the element is usually

Table 1. Mean trace metal concentrations of the various marine food supplements.

Metal	Maximum daily intake	Algae	Coral	Krill	Shark cartilage
(n)		(31)	(5)	(3)	(16)
	(μg/kg/d)		mg/kg		
Cd	1 (WHO 1989)	0.33 ± 0.35^{a}	0.36 ± 0.19^{a}	0.65 ± 0.20^{a}	0.19 ± 0.09^{a}
		(0.016–1.9)	(0.05–0.523)	(0.42–0.82)	(0.039–0.40)
Co	—	0.77 ± 0.86^{a}	0.45 ± 3.5^{a}	0.11 ± 0.03^{a}	0.38 ± 0.13^{a}
		(0.02–4.5)	(0.45–0.77)	(0.09–0.14)	(0.02–0.50)
Cr	3 (USEPA 2005)	3.1 ± 2.9^{a}	3.1 ± 2.9^{a}	0.54 ± 0.08^{a}	3.4 ± 1.4^{a}
		(0.4–11)	(0.45–3.5)	(0.02–0.16)	(0.3–6.3)
Cu	500 (WHO 1989)	6.7 ± 3.3^{a}	3.5 ± 0.5^{a}	63 ± 30^{b}	3.6 ± 0.3^{a}
		(2.7–17)	(2.9–4.1)	(41–97)	(3.0–5.1)
Ni	20(USEPA 2005)	8 ± 15^{a}	3.0 ± 0.8^{a}	0.83 ± 0.06^{a}	1.1 ± 0.3^{a}
		(0.26–73)	(1.7–3.8)	(0.77–0.91)	(0.47–1.8)
Pb	3.57 (WHO 1989)	1.6 ± 1.7^{a}	0.21 ± 0.02^{a}	0.37 ± 0.04^{a}	0.65 ± 0.49^{a}
		(0.28–8.0)	(0.17–0.24)	(0.27–0.40)	(0.25–2.0)
Zn	300 (USEPA 2005)	22 ± 11^{a}	5.1 ± 3.1^{b}	50 ± 10^{c}	37 ± 13^{c}
		(4–47)	(2.8–11)	(42–62)	(2.52–48)
			μg/kg		
Hg	0.71†	10.0 ± 8.5^{a}	1.0 ± 0.5^{a}	13 ± 5^{a}	159 ± 178^{b}
	(WHO 1989)	(0.39–31)	(0.38–1.6)	(7.3–17)	(8.4–538)

Means are given ± standard deviations, means followed by the same letter (a, b, c) for each category of supplements are not statistically different (ANOVA Tukey-b post-hoc test $p < .05$). The range (min-max) of values observed is given within parentheses. †The maximum allowable daily intake for methylmercury is 0.1, albeit we have measured total Hg, for shark cartilage, we expect 95% of the mercury to be methylmercury (Krystek and Ritsema 2005).

0.002–1.0 mg/kg (Kay 1991). Surveys of marine algae or seagrass also showed higher values ranging from 0.10 to 2.80 mg Cd kg^{-1} in one case (van Netten *et al.* 2000) and 0.4 to 3.76 in another study (Campanella *et al.* 2001). In our study, an Atlantic Kelp supplement is also in the high range of those surveys at 1.9 mg/kg, which suggests that high values are not unusual in this category of dietary supplements. The outlier values observed in our work and others suggest that it is worth being careful with Cd levels in seagrass and algae supplements. Cadmium levels in plant or algal tissues do not normally exceed a threshold of 1 mg/kg; plants grown on contaminated soils, like rice and wheat, can, however, have higher cadmium concentrations, sometimes well above the 1 mg/kg limit. Contamination during the processing of the dietary supplements is also a possibility for the high cadmium levels. Our data and the literature show great variability in cadmium concentrations for different food supplements. It is therefore difficult to assess approximate mean values for one specific food supplement category, reinforcing the need for sustained testing of dietary supplements. Given the tendency of algae to accumulate metals (Malik 2005; Martin *et al.* 1997), it is possible that even under marginally contaminated environmental conditions, the algal products could become contaminated.

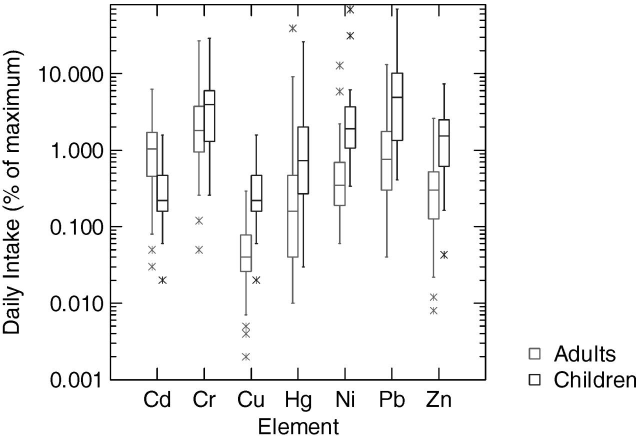

Figure 1. The resulting daily intake are illustrated using box plots (the left boxes represent adults, the right boxes are for children). The center horizontal line marks the median of the samples, the length of each box shows the range within which 50% of the values fall, with the box edges representing the first and third quartiles, the whiskers represent the limits of the inner fences (defined as 1.5* the interquartile range) and outliers are shown as asterisks.

Cadmium intake

For cadmium, as shown in Figure 1, food supplements of marine origin do not usually represent a high dietary source of this metal. Percentages of the PTDI are less than 20% for all the samples in our study. Even the kelp sample with the highest Cd concentration of 1.9 mg/kg gives a Cd ingestion of only 10% of the PTDI for children, less than the maximum intake recommended if the maximum dosage is respected. However, because Cd toxicity is of concern, excessive ingestion of that particular kelp supplement could possibly represent a danger for consumer health, especially for children consumers if the maximum daily exposure is already close to the toxicological limit.

Lead concentrations

The lead contents we measured in our supplements vary from 0.17 to 8.4 mg Pb/kg (Table 1) and albeit the concentrations of Pb in the various supplements are not statistically different (data not shown), in this case, the daily intake of Pb from algae-based products could lead to almost 70% of the tolerable daily intake for children (Figure 1).

An analysis of 15 seaweeds reported concentrations of 0.01 to 0.64 mg Pb/kg (van Netten *et al.* 2000); another analysis of seagrass and brown algae showed levels of 0.23 to 16.9 mg Pb /kg (Campanella *et al.* 2001). Still, large surveys seldom report concentrations of Pb much above 1–2 mg/kg (Phaneuf *et al.* 1999). Another study reports concentrations of 0.1 to 2.1 mg Pb/kg in oyster shell Ca supplements (Scelfo and Flegal 2000). In our dataset, the highest concentrations of lead are in the algae supplements with a mean concentration of 1.6 ± 0.7 mg of Pb kg^{-1} and a maximum level of 8.4 mg/kg for one of the spirulina sample. In fact, the spirulina samples show the highest lead concentrations. In our research, six out of the eight samples even exceed 2 mg/kg. A previous study on trace metals levels in dietary supplements, including marine-based food supplements, has also shown that spirulina supplements contained the highest Pb concentrations, high enough to result in exposure exceeding the tolerable intakes for children and pregnant women (Kay 1991). Thus, spirulina seems to be an alga with a high lead bioconcentration potential and spirulina supplements should be monitored closely for general public safety. For shark cartilages and krill supplements, Pb levels ranged from 0.26–2.6 mg/kg and from 0.27–0.40 mg/kg, respectively, in accordance with Pb concentrations in fish (Mendil *et al.* 2005) and in mussels (Saavedra *et al.* 2004; Scelfo and Flegal 2000). Concentrations of the metal in those studies ranged between 0.7–2.4 mg/kg for fish, 0.75–2.0 mg/kg for wild mussels, and 0.60–1.3 mg/kg for cultivated mussels, very similar with the results in our samples. Coral samples do not represent elevated levels of Pb with a mean concentration of only 0.21 ± 0.02 mg Pb/kg, relatively low when compared to other calcium supplements (Scelfo and Flegal 2000).

Lead intake

For the majority of the samples studied in this experiment, intake of Pb is not important, with many PTDI values below 15%. There is, however, a sample representing a non-negligible source of lead with 70% of the PTDI for children if the maximum daily direction of use is respected. That spirulina sample shows a high Pb level with 5 mg/kg of the contaminant combined with a relatively high dosage (9 tablets daily), providing a daily Pb intake close to the maximum limit fixed by the World Health Organization (WHO 1989). This is cause for concern given that Pb is the element with the most reported cases of poisoning, especially in children, considering that in many urban environments children are already exposed to significant or excessive levels of Pb.

Copper concentrations

Copper concentrations in algae, coral, and shark cartilage are somewhat similar with the levels found in different food products. Statistical tests show that those three categories of marine supplements have Cu concentrations in the same levels (Table 1). Krill supplements, however, have much higher Cu concentrations, between 41 and 97 mg/kg, with a mean of 63 ± 30 mg/kg (Table 3). Nevertheless, those results are not unusual since values of 32–65 mg/kg Cu in krill have been reported previously (Li *et al.* 2005). Levels in seagrass and algae varied between 5.0 to 23.9 mg Cu/kg in samples from the Mediterranean (Campanella *et al.* 2001) and from 0.5 to 8.9 in samples from edible marine algae (van Netten *et al.* 2000).

Copper intake

Copper intake via food supplements of marine origin does not seem to be a major issue. No supplements have an intake exceeding 2% of the PMTDI fixed by the WHO (Figure 1).

Cobalt concentrations and intake

Total Co concentrations in our samples vary from near 0.02 to 4.5 mg Co/kg. Co concentrations are similar across the different supplement categories (data not shown). Except for a few algae outliers, the concentrations are usually below 1 mg Co/kg and hence in the same range reported earlier (van Netten *et al.* 2000). But similarly high concentrations (between 2 and 5 mg Co/kg) have also been reported by others and are certainly not unusual (Phaneuf *et al.* 1999). No intake levels were calculated given the absence of a Co guideline.

Chromium concentrations

Chromium concentrations in our samples are in the range of 0.5–11 mg/kg, within the range of values reported in other studies (Table 1). Levels of Cr in seaweed vary between 0.1 to 2.0 mg Cr/kg but two samples were reported at 11.4 and 11.5 mg Cr/kg (van Netten *et al.* 2000). Seaweed and algae samples from the Mediterranean were between 0.10 and 3.6 mg Cr/kg (Campanella *et al.* 2001).

Chromium intake

Figure 1 shows maximum intake levels approaching 10–30% in algae and shark supplements. Those levels maintain a certain margin of safety suggesting that for the samples we analyzed, there are probably little or no risks associated with the consumption of food supplements of marine origin with respect to Cr. Given the variability it might still be pertinent to verify the seasonal variability of Cr concentrations in new and currently sold products.

Nickel concentrations

Nickel was found in greater concentrations in algae (8 ± 3 mg/kg), followed by coral with 3.1 ± 0.7 mg/kg, krill and then shark cartilages having concentrations of 0.8 ± 0.2 mg/kg and 1.1 ± 0.1 mg/kg, respectively (Table 1 and Figure 1). Biomagnification of Ni in aquatic plants is possible since they do not have the ability to regulate their Ni concentrations like fish and other aquatic animals. Thus, elevated Ni concentrations in algae and coral samples are of no surprise. Most of our samples have Ni concentrations similar or lower than those reported earlier (Martin *et al.* 1997; Phaneuf *et al.* 1999). Some kelp samples have concentrations ranging from 26 mg/kg to 73 mg/kg. Even if the adverse effects of Ni are not well known, ingestion of those four kelp samples should be made with some caution to prevent any adverse effects from excessive nickel exposure.

Nickel intake

In the case of Ni, only two food supplements approach the reference dose fixed at 20 μg/kg/d. Those kelp samples have elevated nickel concentrations, showing 70% and 30% of the TDI for the metal. Toxic effects of Ni are not widely documented but the metal ingested trough food has a tendency to accumulate in the kidneys and in other tissues like bladder, heart, lung, adipose tissues, and brain.

Zinc concentrations

Zinc concentrations in the marine supplements are variable according to the ANOVA test (Table 1) similar to the concentrations reported by various authors (Campanella *et al.* 2001; Martin *et al.* 1997; Phaneuf *et al.* 1999; van Netten *et al.* 2000). Krill is the supplement category having the highest Zn concentration with 50 ± 10 mg Zn/kg, followed by shark cartilage, 37 ± 13 mg/kg, algae, 10 ± 9 mg/kg, and coral, 1.0 ± 0.5 mg/kg.

Zinc intake

Nevertheless, the calculated daily intake of Zn never exceeds 2% of the maximum allowable level—hence there is little or no concern from Zn exposure following the consumption of our supplements (Figure 1).

Mercury concentrations

Total Hg concentrations in all samples ranged between 0.38 and 538 μg of Hg kg^{-1}, with a minimum concentration in the coral samples and maximum levels in the shark cartilage samples (Table 1). The ANOVA post-hoc Tukey-b test shows that Hg concentrations are equivalent in three of the four supplement categories, shark cartilage being the one in which Hg levels are significantly higher. van Netten *et al.* (2000) also report from 50 to 1080 μg Hg kg^{-1} in 15 commercially available marine algae products, but 14 out of their 15 algae were below 440 μg Hg kg^{-1}. Our mean Hg level in shark cartilage is much higher than the value of 60 μg Hg/kg reported for shark cartilage-based Ca supplements (Kim 2004). In the food chain, Hg is biomagnified by different living organisms as it goes up in the food chain due to its long retention time in the tissues. This is why the highest concentrations of Hg found in aquatic organisms are in the larger predatory fish like tuna, swordfish, and shark. In Canada, Hg bioconcentration in those three fish species led the government to put them on a derogatory list for the maximum acceptable methylmercury (MeHg) content of fish, set at 0.5 μg of MeHg per kilogram, because Hg concentrations in those species often exceed this level. Thus, it is not a surprise that the maximum Hg concentrations in our samples have been found in the shark cartilages.

Mercury intake

The relative fraction of intake relative to the PTDI is illustrated in Figure 1 and shows the dietary contribution of Hg from the consumption of food supplements. The consumption of the food supplements in this study at the maximum directions of use does not give a Hg intake exceeding the PTDI given by the WHO/FAO joint committee. Still, of the 12 shark cartilages tested, 2 supplements showed relatively high Hg concentrations. Two samples have Hg concentrations of 532 and 538 μg of Hg kg^{-1}, respectively. Using the maximum direction of use and presuming that all of the Hg is methylated (Krystek and Ritsema 2005), those supplements give a MeHg ingestion of 39% and 40% of the PTDI for adults (Figure 1). For children, shark cartilage supplements also represent the highest potential source of Hg. For most, but not all shark cartilage products, warnings were indicated against the use of the supplements by children. Still, recommendations on two samples of shark cartilage provide no restrictions for use by children and those would represent 25 to 26% of the PTDI. Thus, MeHg intake from marine-based food supplements should not be neglected because the Hg from those supplements has to be considered as an addition to the Hg absorbed through the normal daily diet. Populations with significant fish intakes often have high MeHg ingestion levels and adding shark cartilage supplements to their diet could increase MeHg concentrations in their body to levels representing potential risks for their health.

CONCLUSION

We determined the concentrations of various trace metals in dietary supplements from diverse marine origins. Mercury, Cd, Pb, Co, Cu, Cr, Ni, and Zn levels were measured in algae, coral, krill, and shark cartilage. Shark cartilage supplements were the most concentrated in Hg, given the large predatory status of sharks, we presume that for the most part the Hg in shark cartilage is composed of bioaccumulated methylated Hg (Krystek and Ritsema 2005). Krill supplements showed the highest levels of Cd, Co, and Zn. Lead, Cr, and Ni concentrations were the highest in algae supplements.

In general, occurrence of trace metals in food supplements of marine origins seems to be in the same levels as that occurring in other food products. However, trace metals concentrations can be quite variable within a single supplement category and some of our samples were clear outliers and showed trace metals concentrations above those generally found in foods or in food supplements. For example, the Cd concentration of a kelp supplement was above the 1 mg/kg limit generally fixed for the contaminant in food products. Four samples also showed elevated Ni concentrations, reinforcing the need for sustained quality control tests for those categories of supplements destined for human consumption.

Overall, very few samples approached the PTDI for the trace metals studied. Even if no supplement is exceeding the recognized threshold daily intake for Hg, Cd, Cr, Pb, and Ni, some supplements still represent a significant source of those contaminants. Because trace metal ingestion provided by the consumption of food supplements is added to the normal daily food ingestion, the use of the supplements should be made with some caution and good quality control programs must be emphasized for the safety of those easily accessible supplements.

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REFERENCES

Brownie S. 2005. The development of the US and Australian dietary supplement regulations—What are the implications for product quality. Compl Ther Med 13:191–8

Campanella L, Conti ME, Cubadda F, *et al.* 2001. Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean Environ Pollut 111:117–26

Cubadda F and Raggi A. 2005. Determination of cadmium, lead, iron, nickel and chromium in selected food matrices by plasma spectrometric techniques. Microchem J 79:91–6

Dolan SP, Nortrup DA, Bolger PM, *et al.* 2003. Analysis of dietary supplements for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry. J Agric Food Chem 51:1307–12

Fayad PB, Amyot M, and Sauvé S. 2004. Total mercury determinations in sand boxes from Montreal. J Environ Monit 6:903–6

Feyzi R, Hassan ZM, and Mostafaie A. 2003. Modulation of CD4+ and CD8+ tumor infiltrating lymphocytes by a fraction isolated from shark cartilage: Shark cartilage modulates anti-tumor immunity. Intern Immunopharm 3:921–6

Grollman A. 2005. Academic perspectives on dietary supplements use: The need for new guidelines. Thromb Res 117:185–92

Gulati OP and Ottaway PB. 2006. Legislation relating to nutraceuticals in the European Union with a particular focus on botanical-sourced products. Toxicol 221:75–87

Hagedorn M and Bikfalvi A. 2000. Target molecules for anti-angiogenic therapy: From basic research to clinical trials Crit Rev Oncol/Hemat 34:89–110

Hassan Z, Feyzi R, Sheikhian A, *et al.* 2005. Low molecular weight fraction of shark cartilage can modulate immune responses and abolish angiogenesis. Intern Immunopharm 5:961–70

Kay RA. 1991. Microalgae as food and supplement. Crit Rev Food Sci Nutr 30:555–73

Kim M. 2004. Mercury, cadmium and arsenic contents of calcium dietary supplements. Food Addit Contam 21:763–7

Krystek P and Ritsema R. 2005. Mercury speciation in thawed out and refrozen fish samples by gas chromatography coupled to inductively coupled plasma mass spectrometry and atomic fluorescence spectroscopy. Anal Bioanal Chem 381:354–9

Li B, Bergmann J, Lassen S, *et al.* 2005. Distribution of elements binding to molecules with different molecular weights in aqueous extract of Antarctic krill by size-exclusion chromatography coupled with inductively coupled plasma mass spectrometry. J Chromato B 814:83–91

Malik A. 2005. Metal bioremediation through growing cells. Environ Internat 30:261–78

Martin MH, Nickless G, and Stenner RD. 1997. Concentrations of cadmium, copper, lead, nickel, and zinc in the alga Fucus serratus in the Severn estuary from 1971 to 1995. Chemosphere 34:325–34

Mendil D, Uluözlü ÖD, Hasdemir E, *et al.* 2005. Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey. Food Chem 90:175–9

Nestmann ER, Harwood M, and Martyres S. 2006. An innovative model for regulating supplements products: Natural health products in Canada. Toxicol 221:50–8

Phaneuf D, Côté I, Dumas P, *et al.* 1999. Evaluation of the contamination of marine algae (seaweed) from the St-Lawrence river and likely to be consumed by humans. Environ Res 80:S175–S182

Ritter S. 2004. A fishy tale. Chemical & Engineering News. December 20:80

Rupérez P. 2002. Mineral content of edible marine seaweeds. Food Chem 79:23–6

Saavedra Y, Gonzalez A, Fernandez P, *et al.* 2004. A simple optimized microwave digestion method for multielement monitoring in mussel samples. Spect Acta B: Atom Spect 59:533–41

Scelfo GM and Flegal AR. 2000. Lead in calcium supplements. Environ Health Perspect 108:309–19

Sheu JR, Fu CC, Tsai ML, *et al.* 1998. Effect of U-995, a potent shark cartilage-derived angiogenesis inhibitor, on anti-angiogenesis and anti-tumor activities. Anticancer Res 18:4435–41

Singh S, Kate BN, and Banerjee UC. 2005. Bioactive compounds from cyanobacteria and microalgae: An overview. Crit Rev Biotechnol 25:73–95

Takaichi S, Matsui K, Nakamura M, *et al.* 2003. Fatty acids of astaxanthin esters in krill determined by mild mass spectrometry. Comp Biochem Physiol B Comp Biochem 136:317–22

USEPA (US Environmental Protection Agency). 2005. Integrated Risk Information System IRIS—Revised December 2005. Available at www.epa.gov/iris/gloss8.htm

van Netten C, Cann SA, Morley DR, *et al.* 2000. Elemental and radioactive analysis of commercially available seaweed. Sci Total Environ 255:169–75

WHO (World Health Organization). 1989. An Evaluation of Certain Food Additives and Contaminants. (41st report of the Joint FAO/WHO Expert Committee on food additives). Geneva, Switzerland