

Cancer Risk to Japanese Population from the Consumption of Inorganic Arsenic in Cooked Hijiki

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The cancer risk posed by inorganic arsenic (iAs) ingestion via the consumption of hijiki seaweed, a common Japanese food item known to accumulate pentavalent arsenic, was estimated. Fourteen households were asked to supply three portions of cooked hijiki (boiled and fried with vegetables and fried bean curd, etc.), as usually cooked and served per person in each household. The monthly consumption frequency of cooked hijiki was assessed by questionnaire: it was typically two to three times a month in most households. The mean daily consumption of cooked hijiki was estimated to be 6.5 g/day (range = 1.1–14 g/day, median = 5.5 g/day) by multiplying one serving quantity (grams) by the monthly frequency of consumption. The concentration of iAs [As(III) + As(V)] in the cooked hijiki was determined after homogenization, freeze-drying, 0.07 mol/L HCl extraction, and high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC-ICPMS). The concentration of iAs ranged from 0.4 to 2.8 mg/kg (wet weight basis) in the cooked hijiki, and iAs intake from cooked hijiki was calculated to be 0.0005–0.023 mg/day. On the basis of these data and the oral slope factor [$1.5E0$ (mg/kg/day)⁻¹] reported by the U.S. EPA for iAs, the mean skin cancer risk through cooked hijiki consumption was calculated to be 2.4×10^{-4} (range = 1.6×10^{-6} – 7.0×10^{-4}), which exceeded the acceptable level of 10^{-5} . Taking the risk of other cancers (bladder, lung, etc.) into consideration, the contribution to cancer occurrence through the consumption of hijiki seaweed may not be negligible.

KEYWORDS: Hijiki; inorganic arsenic; cancer risk; oral slope factor

INTRODUCTION

Japanese people consume on a daily basis a variety of seaweeds as a part of their normal diet. The National Nutritional Survey revealed that the average per capita intake of seaweeds in Japan amounted to 14.6 g/day in 2002 (1). Hijiki (*Hizikia fusiforme*) is one such seaweed commonly consumed in everyday food in Japan. Although seaweeds naturally accumulate arsenic (As) as virtually nontoxic arsenosugars (2), hijiki is an exception: it contains toxic inorganic As (iAs) at elevated levels (2–6).

Inorganic As is a known human toxicant, and it has therefore been questioned in the past whether the consumption of hijiki is acceptable from the viewpoint of food safety. Watanabe et al. concluded that the total As intake of Japanese people through the consumption of hijiki was $1/100$ of the minimum dose of As (administered through a hijiki-containing diet) that caused growth retardation in the rat (7). Hanaoka et al. measured the iAs content of cooked hijiki and concluded that iAs intake via

the consumption of cooked hijiki was well below the lethal dose (4). Although iAs is known to be a human carcinogen, the authors did not refer to the cancer risk resulting from the long-term exposure to the iAs in hijiki.

In 2004, the Food Standards Agency (FSA) of the United Kingdom advised on the Web against eating hijiki (8) because it contained iAs. In response to this advice, the Japanese Ministry of Health, Labor, and Welfare (MHLW) announced that the health risk of hijiki consumption was minimal (9) because iAs intake through hijiki consumption was estimated not to exceed the Provisional Tolerable Weekly Intake (PTWI) of 15 $\mu\text{g}/\text{kg}/\text{week}$, proposed by the WHO (10). Almela et al. also discussed the estimated iAs intake via hijiki consumption in relation to the PTWI (5, 6). However, the PTWI is formulated with the purpose of preventing chronic arsenicism and not specifically for cancer prevention. Thus, to date, the MHLW and researchers in the field have, by evaluating iAs intake only in terms of PTWI, failed to evaluate the cancer risk of hijiki consumption.

In this study, we evaluate the cancer risk posed to Japanese people through hijiki consumption by estimating the daily iAs intake from cooked hijiki. The estimation is based on the consumption frequency of, and the concentration of iAs in,

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cooked hijiki. The cancer risk was calculated by multiplying the estimated daily intake of iAs from cooked hijiki and the oral slope factor for iAs proposed by the U.S. EPA. The oral slope factor is an upper bound estimate of human carcinogenic risk (probability) per milligram of agent per kilogram of body weight per day, which was based on the quantitative carcinogenic evaluation of the EPA (11). The oral slope factor for iAs was estimated to be $1.5 \text{ E}0 \text{ (mg/kg/day)}^{-1}$ (12) on the basis of a number of epidemiologic studies (13).

MATERIALS AND METHODS

Cooked Hijiki Samples. After harvest, hijiki seaweed is usually processed by boiling or steaming and is then dried for sale in the Japanese market. The most common recipe for cooking hijiki is to fry and boil it (after reconstitution with water) with vegetables and other foods and to season it with, for example, soy source, sugar, and dried bonito extract ("hijiki no nimono" in Japanese, called cooked hijiki hereafter). It is usually eaten as a side dish.

Fourteen subjects including university students and staff were asked to supply samples of cooked hijiki as eaten in their homes. Hijiki was usually cooked by the housewife, and the recipes differed from household to household in terms of the ingredients that were cooked with the hijiki. The subjects were each asked to supply three portions of cooked hijiki with a portion being that amount usually served to an adult family member in each household. Three portions were required to supply an adequate amount of sample for As analysis and to determine the variability in portion size. The variability of "one serving" was found to be <20%. One additional cooked hijiki was purchased in a supermarket in Chiba prefecture. We had 15 cooked hijiki samples in total.

The frequency with which cooked hijiki is served in each household was assessed by questionnaire.

Sample Preparation. The three portions of cooked hijiki supplied from each household were individually weighed. One of the three portions was randomly selected, and the hijiki was separated from the rest of the ingredients and weighed to give the percentage hijiki in the cooked dish. After weighing, the three serving portions were combined and homogenized in a food processor with a stainless steel blade. We confirmed in a preliminary experiment that there was no iAs contamination from the food processor. A known volume of water (water used in this study was ultrapure from a Millipore Milli-Q apparatus) was added to cooked hijiki for effective homogenization. A portion of the homogenized sample was freeze-dried to constant weight in a 50 mL polypropylene (PP) centrifuge tube.

Inorganic As Extraction. One gram of the freeze-dried cooked hijiki sample was placed in a 50 mL PP tube, and 20 g of a simulated gastric juice was added. The simulated gastric juice was prepared from 0.07 mol/L HCl, prepared from ultrapure grade HCl (AA-100, Tama Chemical Co. Ltd., Kawasaki, Japan), and 20 μL of 10% (w/v) pepsin solution, prepared from porcine pepsin (Sigma-Aldrich, St. Louis, MO). The tube was capped and then horizontally shaken in a water bath at 37 °C for 2 h. The pH of the HCl phase (approximately 1.5) was checked before and after the extraction, but no substantial change occurred in any sample. After centrifugation, the HCl supernatant was transferred to another PP tube and the extraction was repeated twice. The three HCl layers from each cooked hijiki sample were individually stored at 4 °C until analysis. All PP tubes were acid-washed prior to use.

Inorganic As Determination. The iAs concentration in each HCl layer was determined by high-performance liquid chromatography (L-6000, Hitachi High Technologies Co. Ltd., Tokyo, Japan)—inductively coupled plasma mass spectrometry (Agilent 7500ce, Agilent, Tokyo, Japan) (HPLC-ICPMS) with an automatic sample injector (Nanospece SI-2, Shiseido Co. Ltd., Tokyo, Japan). The HPLC column used for As species separation was a Capcell Pak C₁₈ MG S3 ODS (Shiseido Co. Ltd.), and the mobile phase contained 0.004 mol/L malonic acid, 0.004 mol/L tetraethylammonium hydroxide, 0.01 mol/L sodium butanesulfonate, and 0.05% methanol (pH 3) (14). The ICPMS was operated with He as a collision gas (3 mL/min) to reduce $^{40}\text{Ar}^{35}\text{Cl}^+$

interference to ^{75}As . The ion count was monitored at m/z 75 with transient data acquisition mode and at m/z = 77 to detect residual ArCl^+ interference and to correct for it.

Stock standard solutions of As(V), As(III), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AB) at 10 μg As/g were prepared by dissolving appropriate amounts of the following reagents in water: $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, As(III) standard solution for atomic absorption spectrometry and DMA (Kanto Chemical Co. Ltd., Tokyo, Japan), MMA (Tri Chemical Co. Ltd., Yamanashi, Japan), and an AB solution certified reference material (NMIJ CRM 7901-a) from the National Institute of Advanced Industrial Science and Technology, Japan. Working mixed standard solutions (10–100 ng of As/g) were prepared daily by mixing and diluting the stock solutions with water. The iAs concentrations in this paper represent the sums of the concentrations of As(V) and As(III).

The chromatographic condition employed in this study was a modified version of that reported by Shibata and Morita (15), and retention times of major arsenosugars were verified by a cross-check using a PRP X-100 column over seaweed extract sample reported by Madsen et al. (16). Thus, qualitative analysis of major arsenosugars was possible with the present chromatographic condition.

The detection limits were 0.7, 0.5, 0.4, 0.4, and 0.3 ng of As/g for As(V), As(III), MMA, DMA, and AB, respectively, for 10 μL injections. The precision of the analyses was evaluated by repeated measurement of standard solutions (50 ng As/g) and was found to be 2–3% for all five As species when expressed as the relative standard deviation.

Certified reference materials (CRMs), BCR No. 60 Aquatic Plant and BCR No. 279 Sea Lettuce, were analyzed for iAs by our extraction and analysis methods for quality assurance. Note that total As but not iAs concentration was certified in these CRMs. The total iAs concentrations determined by our method [sum of As(V) and As(III) of extractions 1–3] were $5.90 \pm 0.06 \text{ mg of As/kg}$ ($n = 3$) and $1.52 \pm 0.01 \text{ mg of As/kg}$ ($n = 3$) for BCR No. 60 and BCR No. 279, respectively, with good precision. These values compared with literature values of 4.49–4.72 and 1.26–1.37 mg of As/kg, respectively (5, 17). Our method gave 20–30% higher iAs concentration than the published values. This was not due to peak overlap from other major As species in seaweed because As(V) and As(III) were separable from MMA, DMA, AB, and major arsenosugars as mentioned above. We can offer no explanation as to why our method gives higher than the published values.

RESULTS AND DISCUSSION

Cooked Hijiki Consumption. Table 1 shows the weight of one serving (grams per serving), the frequency of consumption (servings per month), and the estimated weight of cooked hijiki per day for each subject. The last was calculated by multiplying the former two and dividing by the number of days in a month (taken to be 30).

Serving size and number of servings varied among the households: on average, 2.4 servings were eaten in a month, and this resulted in a daily consumption of 6.5 g of cooked hijiki. Note that this weight includes other ingredients (carrot, soy bean, deep-fried bean curd, fish paste product, etc.) besides hijiki seaweed. On the basis of the proportion of hijiki seaweed in the cooked hijiki (mean = 50.4%), the average daily hijiki seaweed consumption was estimated to be 3.3 g of wet weight per person. The MHLW estimated the daily hijiki consumption of Japanese people to be 0.9 g on the basis of the National Nutritional Survey data on daily total seaweed consumption (14.6 g) in 2002, with hijiki contributing 6.1% of the total seaweeds sold in the market (9). The present result (3.3 g) was greater than the rough MHLW estimate. One should consider in evaluating this difference that our estimate was based on the actual amount of hijiki eaten by Japanese subjects, although the number of subjects was limited, whereas the MHLW estimate was exclusively based on the national statistics. Both

Table 1. Serving Size, Number of Servings per Month, and Estimated Cooked Hijiki Consumption of the Subjects in This Study

ID	serving size ^{a,c} (g)	no. of servings/month ^b	estimated daily cooked hijiki consumption ^{c,d} (g)
1	68.6	3.0	6.9
2	73.9	2.7	6.6
3	88.6	1.5	4.4
4	89.1	4.5	13.4
5	46.9	2.5	3.9
6	113.5	2.0	7.6
7	145.0	3.0	14.5
8	63.6	0.7	1.4
9	62.1	4.0	8.3
10	47.5	1.5	2.4
11	48.7	0.7	1.1
12	88.8	4.5	13.3
13	81.6	1.0	2.7
14	65.9	2.0	4.4
15	70.0	2.4 ^e	5.6
mean	76.9	2.4	6.4
SD	26.2	1.3	4.3

^a Mean of three serving portions supplied by each household. ^b Assessed by questionnaire. ^c Weight of cooked hijiki including hijiki seaweed and other ingredients. ^d [Estimated daily consumption] = [serving size (g)] × [number of servings/month]/30 (days). ^e ID 15 was purchased, and so there was no information on the number of servings. The mean of the other 14 values was substituted.

of the data have disadvantages; however, in calculating cancer risk, the 4-fold difference may not be substantial. A national consumption survey of hijiki is needed to characterize the toxicity and carcinogenicity of this food.

Qualitative Information on As Species Detected in Cooked Hijiki. Figure 1 shows a typical chromatogram of cooked hijiki extract obtained by HPLC-ICP-MS. As expected, As(V) was the main As species in the cooked hijiki, and As(III) and DMA were detected at lower concentrations. Although MMA was not detected in this chromatogram, it was detected in other samples. Relatively minor peaks from arsenosugars, such as $-\text{OSO}_3$ and $-\text{OPO}_3\text{CH}_2\text{CHOHCH}_2\text{OH}$, were also present in the chromatograms in addition to two unidentified peaks (Figure 1). A minor peak around a retention time of 240 s was considered to be $-\text{SO}_3$ or AB; the retention times of the two compounds were very close in this chromatographic condition. Assignment of peaks of arsenosugars under the present chromatographic condition was done by comparison of retention times of these arsenosugars by using a PRP X-100 column and seaweed extract sample as reported in Madsen et al. (16).

In a previous investigation, inorganic As(V) and $-\text{OSO}_3$ type arsenosugar were found to contribute almost 50% each to the total As in fresh hijiki (3). As shown in this chromatogram, the abundance of $-\text{OSO}_3$ type arsenosugar was much less when compared to the abundance of As(V). This might be due to degradation of the $-\text{OSO}_3$ type during cooking. The unidentified compound at around a retention time of 420 s was assigned as the degradation product of arsenosugars reported by Gamble et al. (18) [designated As(254) in that paper] from a heating experiment of raw hijiki seaweed extract (Narukawa et al., unpublished data).

The presence of As(III), MMA, and, possibly, AB in the cooked hijiki samples may also have resulted from the cooking/acid extraction process. At least a part of the As(III) detected in the extract might have resulted from the reduction of As(V) by HCl during extraction and storage of the extracts. Arsenobetaine, if any, is likely to be from the fish paste product added as an ingredient and/or from the dried bonito extract used for

seasoning. However, to date, there is no literature that indicates production of MMA from the degradation of other As compounds in marine lives. The sources of MMA detected in the present cooked hijiki samples are not known.

Extraction of Inorganic As from Cooked Hijiki. To estimate bioavailable iAs, as well as to consider any changes to the As species that might occur in the stomach, we extracted the bioaccessible fraction with a simulated gastric juice (0.01% pepsin in 0.07 mol/L HCl) rather than attempt to extract total iAs. There were studies that attempted to extract bioaccessible iAs in seaweeds with different simulated gastric juice (mostly diluted HCl with pepsin) and solid-to-liquid ratios (17, 18); however, no procedure has been regarded as standard. Therefore, in this study, we employed simulated gastric juice similar to that of Gamble et al. (18) but without NaCl. We extracted the freeze-dried cooked hijiki powder (1 g) three times with 20 g of the simulated gastric juice. Virtually no As was detected in the second extraction except for a small amount of As(V). Approximately 98% of total extractable As(V) was obtained in the first extract, and the rest was removed by the second extraction. No As(V) was detected in the third extract. All extractable As(III) was found in the first extract and none in the second and third. Thus, it was shown that two 2 h extractions were adequate to remove all of the extractable iAs species. Somewhat lower extractability was observed for the seaweed CRMs analyzed: 82 and 92% of the total iAs for BCR Nos. 60 and 279, respectively, were removed in the first extracts and 96 and 98% in the second. Small amounts of DMA and AB/ $-\text{SO}_3$ were detected in the third extract of the cooked hijiki samples, although they were not detected in the second; this may have resulted from adsorption during the analytical process.

Table 2 shows wet weight concentrations of As species in the cooked hijiki extractable by the simulated gastric juice. The mean iAs concentration [As(III) + As(V)] in the 15 cooked hijiki samples was 1.2 $\mu\text{g/g}$, which was substantially the same as the mean of the sum of the three extractions. Taking into account the moisture content of cooked hijiki (approximately 80%) and the proportion of actual hijiki seaweed in the servings of cooked hijiki (50%), this wet weight concentration was consistent with previously reported iAs concentration in cooked hijiki; for example, Laparra et al. reported bioaccessible iAs in cooked hijiki to be 26 $\mu\text{g/g}$ of dry weight (17). Concentrations of MMA, DMA, and AB/ $-\text{SO}_3$ type arsenosugar were 0.01–0.03 μg of As/g of wet weight and were negligible when compared with that of iAs.

Estimated Daily Inorganic As Intake via the Consumption of Cooked Hijiki. Daily iAs intake through the consumption of cooked hijiki was estimated by multiplying iAs concentrations (micrograms of iAs per gram) and daily cooked hijiki consumption (grams per day; Table 1). The mean and standard deviation of iAs intake were 0.0080 ± 0.0066 mg/day (range = 0.00021–0.023 mg/day, median = 0.0071 mg/day) on the basis of the first extract. The iAs content of one serving of cooked hijiki could be substantial in some cases, being 0.009–0.202 mg/serving (data not shown). Yamauchi et al. carried out a duplicated diet study in Japan, and they reported the mean iAs intake of 35 volunteers to be 0.0337 mg/day with a range of 0.0083–0.101 mg/day (19). The iAs intake through hijiki consumption, 0.008 mg/day on average or up to 0.202 mg per serving, can constitute a significant contribution to the total dietary iAs intake of Japanese people.

The average daily iAs intake, corresponding to 1.1 $\mu\text{g/kg}$ /week (a body weight 50 kg was assumed), was well below the PTWI (15 $\mu\text{g/kg}$ /week). Even the maximum iAs intake of the

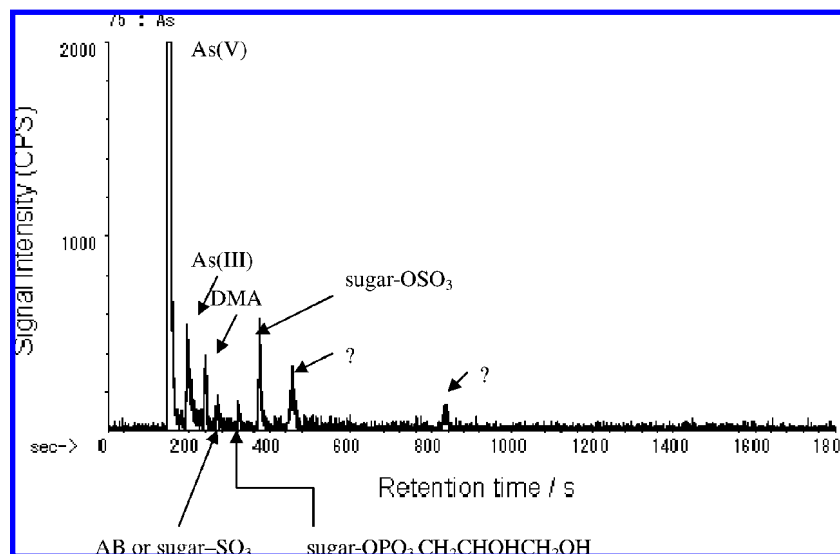


Figure 1. Typical HPLC-ICPMS chromatogram of the 0.07 mol/L HCl extract of cooked hijiki. MMA was not detected in this chromatogram. Peak assignment was based on the analysis of standard solutions for As(V), As(III), MMA, DMA, and AB and comparison of PRP X-100 analysis of seaweed extract provided by Madsen et al. (16) for arsenosugars. Two unidentified peaks were detected in this chromatogram.

Table 2. Mean Concentrations of Bioaccessible As Species (Micrograms of As per Gram of Wet Weight) in Cooked Hijiki Samples ($n = 15$)

	first extract	sum of extracts 1-3
As(V)	1.2 (0.7)	1.2 (0.8)
As(III)	0.031 (0.014)	0.031 (0.014)
inorganic As	1.2 (0.7)	1.2 (0.8)
MMA	0.010 (0.006)	0.010 (0.006)
DMA	0.027 (0.025)	0.030 (0.026)
AB/ $-SO_3$ type arsenosugar	0.027 (0.027)	0.028 (0.027)

15 subjects (0.023 mg/day or 3.2 μ g/kg/week) was below the PTWI. This result indicated that dietary intake of iAs via the consumption of cooked hijiki alone did not exceed the PTWI as estimated by the MHLW (9).

Cancer Risk via the Consumption of Cooked Hijiki. We calculated the excess cancer risk through the consumption of cooked hijiki by multiplying the estimated daily intake of iAs by the oral slope factor [$1.5 \text{ E}0 \text{ (mg/kg/day)}^{-1}$] proposed by the U.S. EPA for iAs. A body weight of 50 kg was assumed for the average Japanese person in the calculation. The mean excess lifetime cancer risk derived from the consumption of cooked hijiki was 2.4×10^{-4} , with the range being from 6.2×10^{-6} to 7.0×10^{-4} . The value was almost identical whether the amount of iAs in first extract or the total iAs in the three extracts was used for calculation. It is generally recognized that the acceptable lifetime cancer risk of a carcinogenic agent is 10^{-5} – 10^{-6} ; the cancer risk posed by hijiki consumption exceeds this acceptable level by a factor of 10. Even if we adopt per capita hijiki consumption of the MHLW estimate (0.9 g/day), the cancer risk would be 6.0×10^{-5} and still exceed the acceptable level. The mean 2.4×10^{-4} risk of our estimate corresponds to an annual incidence of 400 skin cancer cases. Approximately 8000 skin cancer (basal cell and squamous cell carcinoma) cases are recorded annually in Japan. The calculated excess cancer risk due to iAs via cooked hijiki consumption of the subjects in the present study was therefore not negligible. We have no reason to suppose that our participants' hijiki consumption differed from the national average.

The oral slope factor of the U.S. EPA was based on the iAs dose–response for skin cancer incidence in Taiwan and is an upper bound estimate. Thus, the present estimate of 2.4×10^{-4}

risk or an annual skin cancer incidence of 400 should be an overestimation of the actual risk/incidence. However, the present estimate might be considered an underestimation because iAs is considered to be responsible for other cancers besides skin cancer, such as those of the lung and bladder (20). Therefore, the actual total cancer risk through hijiki consumption may be greater than our estimate.

It should be pointed out, though, that the present cancer risk estimate was based upon two major assumptions. First, that the incidence of cancer is a linear function of iAs intake in a low-dose range; in other words, the mechanism of carcinogenesis by iAs was assumed to have no threshold dose. However, some research has indicated there is a threshold in carcinogenesis caused by iAs (21). If this is the case, the present cancer risk estimation based on the oral slope factor would not be appropriate. Further knowledge of the mechanism of carcinogenesis by iAs is necessary to refine information on the cancer risk posed by the consumption of hijiki by Japanese people. The second assumption was that averaging the iAs intake via the intermittent consumption of cooked hijiki (two or three times a month) to give a daily intake value for cancer risk calculation was valid. This assumption may partly be justified by the fact that the biological half-time of ingested iAs in humans is estimated to be 4 days (20). However, the assumption may result in an overestimation of the risk to some extent.

The MHLW of Japan stressed possible health benefits of hijiki consumption (9). Hijiki and other edible seaweeds contain high concentrations, and thus can be a rich source, of dietary fiber and minerals (e.g., calcium, iron, and iodine). This health benefit has also to be quantified to allow the public to judge whether the cancer risk of eating hijiki seaweed is acceptable.

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