Regulatory Toxicology and Pharmacology 63 (2012) 291–297

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Toxicity of so-called edible *hijiki* seaweed (*Sargassum fusiforme*) containing inorganic arsenic

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A R T I C L E I N F O

Article history:
Received 18 November 2011
Available online 27 April 2012

Keywords:
Inorganic arsenic
Toxicity
Rats
Poisoning
High body temperature
Alkaline phosphatase

A B S T R A C T

The UK Food Standards Agency and its counterparts in other countries have warned consumers not to eat *hijiki* (*Sargassum fusiforme*; synonym *Hizikia fusiformis*), a Sargasso seaweed, because it contains large amounts of inorganic arsenic. We investigated dietary exposure of *hijiki* in weaning male F344/N rats fed an AIN-93G diet supplemented with 3% (w/w) *hijiki* powder for 7 weeks, compared with those fed only an AIN-93G diet. Body weight, body temperature, blood and tissue arsenic concentrations, plasma biochemistry and hematological parameters were measured. We found that feeding rats a 3% *hijiki* diet led to a marked accumulation of arsenic in blood and tissues, and evoked a high body temperature and abnormal blood biochemistry including elevated plasma alkaline phosphatase activity and inorganic phosphorus, consistent with arsenic poisoning. These findings should prompt further investigations to identify the health hazards related to consumption of *hijiki* and related *Sargassum* species in humans.

1. Introduction

Exposure to inorganic arsenic is an environmental concern (Islam et al., 2004; Polizzotto et al., 2008; Pringle, 2009). Excessive consumption of arsenic is known to cause various diseases including cancer (Kitchin and Conolly, 2010), impairment in reproduction (Ahmad et al., 2001; Rahman et al., 2010) and sperm count in males (Li et al., 2012), and atherosclerosis (Hsieh et al., 2011). Inorganic arsenic exposure induces abnormalities in biochemical and physiological parameters, including the accumulation of arsenic (Mandal et al., 2007), elevated plasma inorganic phosphorus and alkaline phosphatase (Nabi et al., 2005; Wang et al., 2006), increased total cholesterol (Aguilar et al., 1997) and high body temperature (Saxena et al., 1991; Wilkinson et al., 1975).

Sargasso seaweeds (i.e., genus *Sargassum*), which taxonomically include *hijiki*, contain high levels of inorganic arsenic that account for 80% of the total arsenic content (Sanders, 1979), partly due to phytochelatins (Gekeler et al., 1988) that sequester inorganic arsenic (Perales-Vela et al., 2006). Sargasso seaweeds are usually not considered edible in Western countries. However, ancient Chileans and contemporary Japanese consider them edible. In Chile, Sargasso seaweeds were traditional medicines or foods (Dillehay et al., 2008), which might explain elevated arsenic levels in prehistoric Chilean mummies (Pringle, 2009). On the opposite side of the Pacific Ocean, people in Japan traditionally consume Sargasso seaweeds including *hijiki*, which could account for an eightfold higher urinary level of inorganic arsenic in Japanese than in Americans or Italians (Hata et al., 2007).

In Western countries, *hijiki* is available in natural food stores and sushi bars. In the US, a case of suspected arsenic poisoning due to an herbal kelp supplement has been reported (Amster et al., 2007). Since kelp does not contain a significant amount of inorganic arsenic but *hijiki* does (McGuffin and Dentali, 2007), contamination of *hijiki* in the herbal products might be possible. In 2001, the Canadian Food Inspection Agency (2001) advised the public not to eat *hijiki*. In 2004, the UK Food Standards Agency (2004) issued a warning not to eat *hijiki*. This alert was reissued in 2010 (UK Food Standards Agency, 2010). The European Commission (2004) discussed further action for *hijiki* in addition to the risk alert. Food Standards Australia-New Zealand (2004) and the Hong Kong Centre for Food Safety (2005) advised the public not to eat *hijiki* and banned imports and sales of *hijiki*. The Japanese Ministry of Health, Labour and Welfare (2004) advised consumers not to eat "too much" *hijiki*, i.e., less than 4.7 g daily as consumed after preparation (soaking and removing excess water).

In contrast to this alert, the Japanese Ministry also identified *hijiki* as a good source of dietary fiber and essential minerals (Japanese Ministry of Health, Labour and Welfare, 2004). The

Abbreviations: BW, body weight; JECFA, Joint FAO/WHO Expert Committee on Food Additives; PTWI, provisional tolerable weekly intake.

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http://dx.doi.org/10.1016/j.yrtph.2012.04.006

Japanese Food Guide illustrated this dish, showing 70 g of simmered *hijiki* with soybeans as an amount for one serving (Japanese Food Guide Committee Interim, 2005), and the Japanese Ministry of Health, Labour and Welfare (2002) advised pregnant and puerperal women to eat *hijiki* seaweed for preventing anemia.

Ichikawa et al. (2010) reported that 5% of the ingested arsenic in *hijiki* was retained in the bodies of mice. Japanese researchers carried out a study using a 42-year-old male volunteer, and found that one serving of commercially available processed *hijiki* product containing 22.7 g of soaked and cooked *hijiki* gave a urinary arsenic excretion similar to that in arsenic poisoning (Nakajima et al., 2006). In their review, Tsuji et al. (2007) mentioned a possible exposure of US subpopulations to inorganic arsenic through eating *hijiki*.

Evaluation of the toxicity of *hijiki per se* is crucial to protect people from inorganic arsenic exposure from this seaweed by an appropriate regulatory action, although it has not been tested. In the present study, we evaluated whether ingestion of *hijiki* causes arsenic poisoning using a rat model.

2. Materials and methods

The present study was approved by the Ethical Committee for Laboratory Animals of Seitoku University, Matsudo, Chiba, Japan. All animal care guidelines and animal handling procedures followed were concordant with the Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, 1980, The Japanese Prime Minister's Office).

Twenty weaning male F344/N rats (Japan SLC, Shizuoka, Japan) were divided equally into control (CON) and *hijiki* (HIJ) groups. CON was given an AIN-93G diet (Reeves et al., 1993), while HIJ was given a 3% *hijiki* diet that contained 30 g commercial *hijiki* powder/kg diet, replacing the same amount of cornstarch. Animals were freely given the diets and deionized water for 7 weeks at 22 °C and 50% relative humidity with a 12 h light/dark cycle. At the end of feeding, rectal temperature was measured by a thermistor probe inserted approx. 3 cm from the anus into the rectum (Thermal Sensor; Shibaura Electronics, Saitama, Japan). Skin temperature was measured by an infrared thermometer (IT-550S, Horiba, Kyoto, Japan) at the dorsal side of the tail base, and at the center of the ventral side of the right and left ear auricles. Food was then withheld for 16 h, and blood was collected in a heparinized syringe from the abdominal aorta under diethyl ether anesthesia.

Blood and tissues, *hijiki* powder, basal diets and Standard Reference Material 1548a Typical Diet (NIST, Gaithersburg, MD, USA) were ashed using nitric acid and hydrogen peroxide. Inorganic arsenic in the *hijiki* powder and basal diets was extracted, back-extracted and ashed according to the method of Munoz et al. (2000). Arsenic concentrations were measured by inductively coupled plasma–mass spectrometry (ICPM-8500, Shimadzu, Kyoto, Japan). The analyzed value of the Typical Diet was 0.21 μg arsenic/g, whereas the certified value was 0.20 μg arsenic/g, with a 95% confidence interval of 0.01 μg arsenic/g.

Plasma biochemistry including concentrations of fatty acids, triglycerides, total cholesterol, low-density lipoprotein–cholesterol, phospholipids, inorganic phosphorus, calcium, magnesium, iron, creatinine, uric acid, urea nitrogen, triiodothyronine and thyroxin, and activities of alkaline phosphatase, choline esterase, alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transpeptidase was done by Matsudo Medical Laboratories, Matsudo, Chiba, Japan.

Hematological parameters (red cell counts, white cell counts, platelet counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were measured by an automatic hematological analyzer (Celltac MEK-5258, Nihon Kohden, Tokyo, Japan).

All data were statistically analyzed by the Mann–Whitney test (Mann and Whitney, 1947). *P* values (two-tailed) less than 0.05 were considered significant.

3. Results

The total arsenic content of the *hijiki* powder was 101.8 ± 1.6 μg/g (mean ± SD, $n = 5$) and the inorganic arsenic content was 86.6 ± 5.6 μg/g (mean ± SD, $n = 7$). The inorganic arsenic content was 85.1% of the total arsenic content. These values were similar to the average arsenic levels (i.e., total arsenic 109 μg/g and inorganic arsenic 77 μg/g) found in retail samples of dried *hijiki* within the UK (Rose et al., 2007). Total arsenic content in the basal diet was 15.5 ± 0.8 ng/g (mean ± SD, $n = 5$). Inorganic arsenic was not detected in the basal diet, where the detection limit of inorganic arsenic was 7 ng/g.

The initial body weights (mean ± SD) were 51.4 ± 3.6 g ($n = 10$) and 51.0 ± 4.4 g ($n = 10$) for rats in the control (CON) and *hijiki* (HIJ) groups, respectively. Rats in CON and HIJ were given an AIN-93G diet or 3% *hijiki* diet for 7 weeks, respectively. The final body weights (BW) were 237.5 ± 12.8 g and 239.5 ± 15.0 g for CON and HIJ, respectively. Food intake during the experimental period was 631 ± 39 g and 678 ± 43 g for CON and HIJ, respectively. Energy consumption was 1832 ± 114 kcal and 1899 ± 119 kcal for CON and HIJ, respectively. *Hijiki* consumption significantly increased food intake ($P < 0.01$), while it did not affect energy consumption. The cumulative intake of total arsenic and inorganic arsenic was 10 μg and <4 μg for CON, respectively. The cumulative intake of total arsenic and inorganic arsenic was 2.07 mg and 1.46 mg for HIJ, respectively.

Rats that were given a 3% *hijiki* diet exhibited hair loss after 5 weeks on the dietary regimen. The rectal temperature of rats in HIJ measured after the dietary regimen was significantly higher than that in CON ($P < 0.001$). Skin temperatures were not significantly different between the two groups (Table 1). Consumption of *hijiki* caused markedly elevated arsenic concentrations in blood and tissues including brain, heart, liver, adrenal, testis and tibia (Table 2). The elevations in arsenic seen in HIJ compared with CON were significant at $P < 0.01$ for blood and all organs.

The plasma levels of free fatty acids, triglycerides and total iron binding capacity in HIJ were significantly lower than those in CON. The rats in HIJ had significantly higher plasma levels of total cholesterol, low-density lipoprotein-cholesterol, phospholipids, alkaline phosphatase activity, choline esterase activity, inorganic phosphorous and magnesium than the rats in CON (Table 3). *Hijiki* consumption did not affect calcium, albumin, total protein, alanine aminotransferase activity, aspartate aminotransferase activity, γ-glutamyl transpeptidase activity, uric acid, urea nitrogen,

Table 1
Body temperatures (°C) of rats fed a control diet or 3% *hijiki* diet.

	Control group	*Hijiki* group
Rectal temperature	37.28 ± 0.10 (10)	37.78 ± 0.18** (10)
Skin temperature at the dorsal side of the tail base	31.78 ± 1.68 (10)	33.11 ± 2.10 (10)
Skin temperature at the center of the ventral side of the right ear auricle	32.21 ± 0.67 (10)	31.84 ± 1.11 (10)
Skin temperature at the center of the ventral side of the left ear auricle	32.09 ± 0.69 (10)	31.89 ± 1.28 (10)

Data are mean ± SD (*n*). Double asterisks (**) denote $P < 0.01$ compared with the control group (Mann–Whitney test, two-tailed).

Table 2
Arsenic concentrations in blood and tissues of rats fed a control diet or 3% *hijiki* diet.

		Control group	*Hijiki* group
Blood	μg/g	0.177 ± 0.011 (9)	67.6 ± 2.7** (7)
Brain	ng/g	5.3 ± 1.5 (10)	799 ± 96** (10)
Heart	μg/g	0.012 ± 0.004 (9)	1.60 ± 0.45** (10)
Liver	μg/g	0.012 ± 0.012 (10)	3.17 ± 0.57** (10)
Adrenal	μg/g	0.029 ± 0.045 (9)	2.23 ± 0.92** (8)
Testis	ng/g	2.3 ± 0.7 (10)	649 ± 83** (6)
Tibia	μg/g	n.d. (10)	2.45 ± 0.58** (10)

Data are mean ± SD (*n*). Double asterisks (**) denote $P < 0.01$ compared with the control group (Mann–Whitney test, two-tailed). n.d., not detected (<12 ng/g).

Table 3
Plasma biochemical parameters of rats fed a control diet or 3% *hijiki* diet.

		Control group	*Hijiki* group
Albumin	g/dL	2.89 ± 0.11	2.83 ± 0.08
Total protein	g/dL	6.44 ± 0.21	6.28 ± 0.18
Free fatty acids	mEq/L	1.42 ± 0.22	1.12 ± 0.16**
Triglycerides	mg/dL	51.9 ± 19.4	35.4 ± 21.6*
Total cholesterol	mg/dL	51.3 ± 4.9	61.6 ± 5.4**
Low-density lipoprotein-cholesterol	mg/dL	29.4 ± 3.6	37.3 ± 4.4**
Phospholipids	mg/dL	86.2 ± 5.8	94.1 ± 7.1*
Alanine aminotransferase	U/L	31.7 ± 1.6	32.0 ± 2.5
Aspartate aminotransferase	U/L	72.2 ± 4.6	72.0 ± 6.7
γ-Glutamyl transpeptidase	U/L	n.d.	n.d.
Alkaline phosphatase	U/L	706 ± 30	761 ± 53*
Choline esterase	U/L	73.9 ± 5.9	86.5 ± 12.5*
Inorganic phosphorus	mg/dL	6.36 ± 0.40	6.93 ± 0.59*
Magnesium	mg/dL	1.70 ± 0.09	1.93 ± 0.20**
Calcium	mEq/L	5.54 ± 0.15	5.48 ± 0.17
Uric acid	mg/dL	3.28 ± 0.59	3.51 ± 0.56
Urea nitrogen	mg/dL	13.9 ± 1.0	14.2 ± 1.2
Creatinine	mg/dL	0.15 ± 0.02	0.16 ± 0.02
Iron	μg/dL	118 ± 24	117 ± 26
Total iron biding capacity	μg/dL	594 ± 32	551 ± 22**
Triiodothyronine	ng/dL	64.9 ± 9.5	65.4 ± 7.9
Thyroxin	μg/dL	4.1 ± 1.4	4.9 ± 0.5

Data are mean ± SD ($n = 10$ each). Single asterisks (*) denote $P < 0.05$, and double asterisks (**) denote $P < 0.01$ compared with the control group (Mann–Whitney test, two-tailed). n.d., not detected.

Table 4
Hematological parameters of rats fed a control diet or 3% *hijiki* diet.

		Control group	*Hijiki* group
White blood cell counts	$10^3/\mu l$	4.27 ± 0.67	4.26 ± 0.65
Red blood cell counts	$10^6/\mu l$	8.20 ± 0.30	8.15 ± 0.40
Hemoglobin concentration	g/l	154 ± 4	155 ± 4
Hematocrit	%	57.0 ± 1.6	57.9 ± 1.6
Mean corpuscular volume	fl	57.0 ± 1.6	57.9 ± 2.0
Mean corpuscular hemoglobin	pg	18.8 ± 0.4	19.0 ± 0.7
Mean corpuscular hemoglobin concentration	g/l	331 ± 8	329 ± 6
Platelet counts	$10^3/\mu l$	678 ± 53	659 ± 32

Data are mean ± SD ($n = 10$ each). Data were compared by the Mann–Whitney test (two-tailed) and no significant difference was found.

creatinine, iron, triiodothyronine, and thyroxin in plasma. Hematological parameters were not different between CON and HIJ (Table 4).

4. Discussion

We found various changes in tissue arsenic levels, body temperature and plasma biochemistry accompanying *hijiki* consumption in rats. Our results confirm those reported by other investigators who studied the toxicity of inorganic arsenic (Aguilar et al., 1997; Mandal et al., 2007; Nabi et al., 2005; Saxena et al., 1991; Wang et al., 2006; Wilkinson et al., 1975).

The safety limit for inorganic arsenic for food is currently being revised. In 2010, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) determined the inorganic arsenic benchmark dose lower confidence limit for a 0.5% increased incidence of lung cancer in humans (BMDL0.5). The BMDL0.5 was computed to be 3.0 μg/kg BW/day (2–7 μg/kg BW/day). Therefore, the JECFA withdrew the Provisional Tolerable Weekly Intakes (PTWI) of 15 μg/kg BW/week of inorganic arsenic established in 1989, since the previous PTWI (15 μg/kg BW/week = 2.1 μg/kg BW/day) is similar to the BMDL0.5 of 2–7 μg/kg BW/day (World Health Organization, 2011).

The JECFA summarized the total diet study on inorganic arsenic exposure (World Health Organization, 2011) and reported that the 95th percentile exposure of inorganic arsenic was 0.83–1.29 μg/kg BW/day for Japanese adults and 0.61–2.66 μg/kg BW/day for European children aged 1–8 years, and the mean exposure of inorganic arsenic was 2.09–21.48 μg/kg BW/day for Chilean adults and 1.68–3.00 μg/kg BW/day for Bangladeshi adults. Kile et al. (2007) surveyed inorganic arsenic intake from diet and water in 47 Bangladeshi women and found that 34% of all participants exceeded the previous PTWI (2.1 μg/kg BW). It is evident that the exposure limits of arsenic are being exceeded in some populations.

At the 27th meeting of the JECFA held in 1983, the Committee concluded that an inorganic arsenic intake of 1.5 mg/day was likely to result in chronic arsenic toxicity, and that a daily intake of 0.15 mg may also be toxic in the long term to some individuals (World Health Organization, 2011). The amount of tested *hijiki* in a 3% *hijiki* diet corresponded to 15 g/d for humans, assuming the daily food intake is 500 g dry weight. The amount of inorganic arsenic in 15 g of the *hijiki* tested was 1.3 mg, which is close to the level of chronic arsenic toxicity assessed by the 27th meeting of the JECFA. Considering a maximum (default) interspecies extrapolation factor of 10, 1.5 g of tested *hijiki* containing 0.13 mg inorganic arsenic per day is potentially toxic to humans.

Currently, dried *hijiki* powder is available via retailers including Internet shopping, and eating dried *hijiki* powder is not uncommon in Japan (Okabe et al., 2010; Tsutsui and Kanai, 2002). According to the report to the Japanese Food Safety Commission by the Mitsubishi Chemical Safety Institute (2007), inorganic arsenic intake from commercial *hijiki* powder supplements was estimated to be 29.8–59.7 μg As/kg BW/week or 4.26–8.53 μg As/kg BW/day for persons taking *hijiki* powder supplements in accordance with the manufacturer's instructions. Klei and Barchowsky (2008) found a strong positive interaction between arsenic and ethanol for angiogenic gene induction in human microvascular endothelial cells as a mechanism of atherosclerosis caused by arsenic. Eating foods high in inorganic arsenic such as *hijiki* with alcohol (in any form) is ill-advised if one is to avoid arsenic toxicity. Further, a dose–response study is needed to determine a threshold for the detrimental effects of *hijiki*, with or without concomitant alcohol.

Japanese seaweeds available in the retail outlets are currently limited. Fig. 1 shows the commercially available common Japanese dried seaweeds. *Hijiki* contains excessive amounts of arsenic, while the other seaweeds *arame*, *kombu*, *wakame*, *nori* and *aosa* contain insignificant amounts of inorganic arsenic (Almela et al., 2006; Rose et al., 2007).

In addition to *hijiki*, other Sargasso seaweeds containing high levels of inorganic arsenic are used to make some foods in Japan. Arsenic content in Sargasso seaweeds and related species is summarized in Table 5. *Sargassum horneri* ("*akamoku*" in Japanese) is used for making some types of noodles (Katayama et al., 2008); *Sargassum fulvellum* ("*hon'dawara*" in Japanese) is used today for

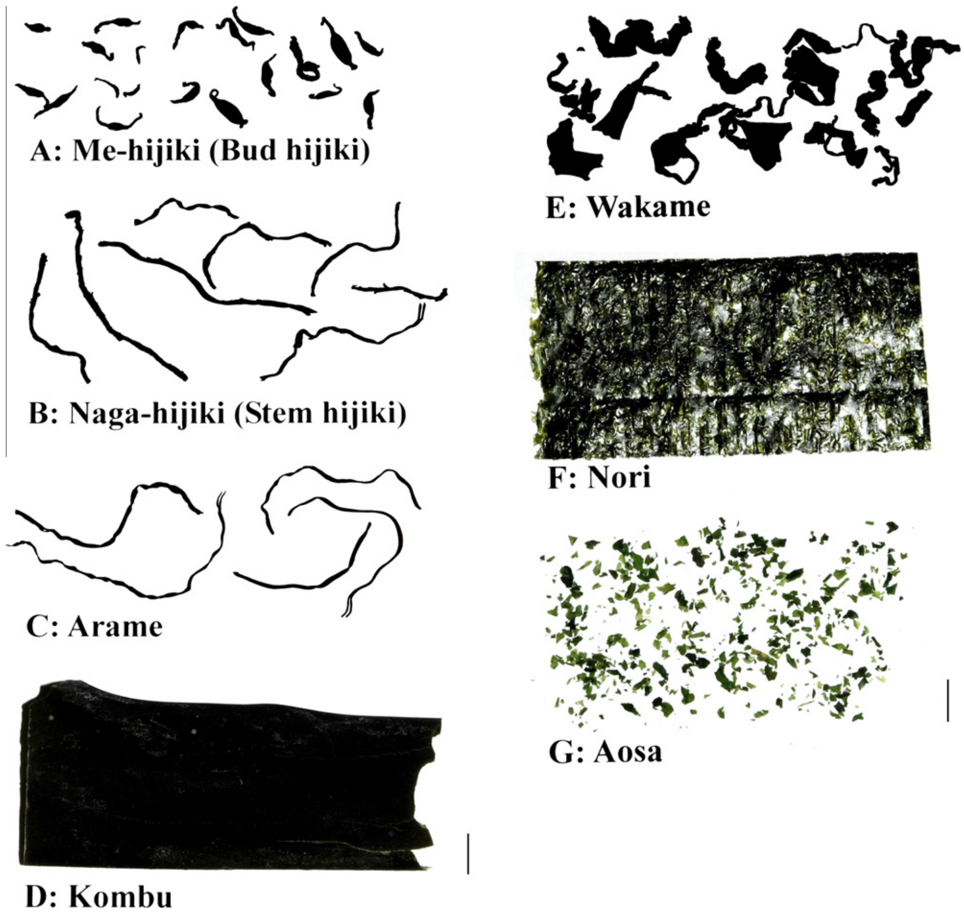

Fig. 1. Common Japanese dried seaweeds commercially available as food. The scale bar shows 1 cm. (A) *Me-hijiki* is the dried buds and leaves of the brown algae *Sargassum fusiforme*. (B) *Naga-hijiki* is the dried stems of *S. fusiforme*. (C) *Arame* is the brown algae *Eisenia bicyclis* (synonym *Ecklonia bicyclis*), dried and cut into thin strands. (D) *Kombu* is a generic name for the Japanese brown algae *Saccharina* species and relatives that include *ma-kombu* (*Saccharina japonica*; synonym *Laminaria japonica*) shown here. (E) *Wakame* is the brown algae *Undaria pinnatifida*. (F) *Nori* is a generic name for the red algae *Pyropia* or *Porphyra* species. Low- to moderate-grade *nori* is usually made from cultivated varieties based on *Pyropia yezoensis* (synonym *Porphyra yezoensis*) (Shiokawa, 2008). The specimen shown here was lightly roasted and it then turned greenish; otherwise it is purplish-black. G: *Aosa* is a generic name for the green algae *Ulva pertusa* and related species.

Table 5
Arsenic content (µg/g dry weight) in *hijiki*, other *Sargassum* seaweeds and related species. Each row corresponds to one specimen for the respective species.

Scientific name (Japanese name) part of seaweed if designated	Origin	As(III)	As(V)	Inorganic As	Total As	References
Sargassum confusum (Fushisujimoku)	Hokkaido, Japan	1.4	53.7	55.1	72	Jin (1983)
		–	–	–	58	
Sargassum fallax[a]	South Australia	–	–	1.7 (1.0–3.8)[g]	79 (64–123)[g]	Maher (1983)
Sargassum fallax[a]	South Australia	–	–	–	125	Maher and Clarke (1984)
		–	–	–	58.4	
Sargassum filipendula	Gulf of Mexico	0.91[h]	4.3[h]	5.21[h]	5.8[h]	Johnson and Braman (1975)
Sargassum filipendula[b]	Florida, USA	–	–	25.3[i]	31.6	Sanders (1979)
Sargassum fluitans	Bermuda	1.8[h]	17.7[h]	19.5[h]	19.5[h]	Johnson and Braman (1975)
Sargassum fulvellum (Hon'dawara)	Shizuoka, Japan	0	72	72	113	Garcia Salgado et al. (2006)
Sargassum fusiforme[c] (*Hijiki*)	Hokkaido, Japan	8.7	48.0	56.7	105	Jin (1983)
		–	–	–	114	
		–	–	–	110	
Sargassum fusiforme[d] (Hijiki)	Japan	–	–	71.8	134.1	Whyte and Englar (1983)
Sargassum fusiforme[c] (*Hijiki*)	Japan	–	–	73	107	Rose et al. (2007)
		–	–	80	112	UK Food Standards Agency (2004)
		–	–	83	116	
		–	–	69	100	
		–	–	67	95	
		–	–	81	110	
		–	–	76	112	
		–	–	72	102	
		–	–	96	124	

Table 5 (*continued*)

Scientific name (Japanese name) part of seaweed if designated	Origin	As(III)	As(V)	Inorganic As	Total As	References
Sargassum fusiforme[d] (*Hijiki*)	Japan	–	–	34	54	McSheehy and Szpunar (2000)
		–	–	22	45	
Sargassum fusiforme[d] (*Hijiki*)	Japan	–	–	75.4	111	Almela et al. (2006)
		–	–	41.6	89.2	
		–	–	91.2	114	
		–	–	81.1	131	
		–	–	61.6	93.9	
		–	–	80.3	124	
		–	–	117	149	
		–	–	43.7	68.3	
		–	–	69.4	106	
Sargassum fusiforme[e] (*Hijiki*) bud and leaf	Japan	1.5	32.0	33.5	41.7	Ichikawa et al. (2006)
		10.3	29.0	39.3	44.4	
Sargassum fusiforme[e] (*Hijiki*) stem	Japan	0.7	36.8	37.5	45.8	Ichikawa et al. (2006)
		12.6	25.0	37.6	46.7	
Sargassum fusiforme[e] (*Hijiki*) bud and leaf	South Korea	1.3	60.5	61.8	71.5	Ichikawa et al. (2006)
		4.0	51.2	55.2	65.6	
Sargassum fusiforme[e] (*Hijiki*) stem	South Korea	n.d.	66.8	66.8	79.5	Ichikawa et al. (2006)
		n.d.	69.0	69.0	79.8	
Sargassum fusiforme[e] (*Hijiki*) bud and leaf	China	n.d.	38.8	38.8	48.6	Ichikawa et al. (2006)
		n.d.	30.7	30.7	36.0	
Sargassum fusiforme[e] (*Hijiki*) stem	China	n.d.	32.1	32.1	37.5	Ichikawa et al. (2006)
		n.d.	32.4	32.4	42.4	
Sargassum fusiforme[d] (*Hijiki*) bud and leaf	Yamaguchi, Japan	–	–	–	231.0	Hanaoka et al. (2001)
Sargassum fusiforme[d] (*Hijiki*) stem	Yamaguchi, Japan	–	–	–	91.2	Hanaoka et al. (2001)
Sargassum horneri (Akamoku) twig	Mie, Japan	–	–	–	114.3[j]	Katayama et al. (2008)
		–	–	–	144.8[k]	
Sargassum horneri (Akamoku) stem	Mie, Japan	–	–	–	59.7[j]	Katayama et al. (2008)
		–	–	–	51.4[k]	
Sargassum horneri (Akamoku)	Miyagi, Japan	–	–	–	92.1	Suzuki and Iwata (1990)
Sargassum linearifolium	South Australia	–	–	–	58.4	Maher and Clarke (1984)
Sargassum miyabei (Miyabemoku)	Hokkaido, Japan	6.4	57.5	61.9	96	Jin (1983)
Sargassum muticum (Tamahahakimoku)	British Columbia, Canada	–	–	20.8	54.8	Whyte and Englar (1983)
Sargassum thunbergii (Umitoranoo)	Hokkaido, Japan	3.05	67.7	70.75	109	Jin (1983)
Stephanocystis hakodatensis (Uganomoku)	Hokkaido, Japan	2.6	40.6	43.2	320	Jin (1983)
		0.94	61.6	62.54	140	
Stephanocystis osmundacea[f]	California, USA	–	–	28.4[h]	–	Andreae (1978)

–: not analyzed.
[a] Reported under the synonym *Sargassum bracteolosum*.
[b] Reported under the misnomer *Sargassum filapendulum*.
[c] Reported under the Japanese name *Hijiki* or *Hiziki*.
[d] Reported under the synonym *Hizikia fusiforme*.
[e] Reported under the misnomer *Hijikia fusiforme*.
[f] Reported under the synonym *Cystoseira osmundacea*.
[g] Range (minimum–maximum).
[h] Wet weight.
[i] Shown as 80% of total arsenic.
[j] Female.
[k] Male.

making a kind of sea salt called "*moshio*" in Japanese (Tomioka, 2000), although traditional materials for "*moshio*" production are eelgrass, *Zostera marina* ("*amamo*" *or* "*moshiogusa*" *in Japanese*), and related species that do not accumulate arsenic like Sargasso seaweeds (Howley et al., 2004; Komoroske et al., 2012; Maher et al., 2011). *Sargassum fulvellum* is already proven to contain a high amount of inorganic arsenic, while the inorganic arsenic content in *Sargassum horneri* has not been analyzed. Considering that seaweed species belonging to the genus *Sargassum* generally contain extremely high amounts of inorganic arsenic (Table 5), any *Sargassum* seaweeds need to be handled cautiously.

It is of interest that seaweeds containing high levels of inorganic arsenic are not limited to the genus *Sargassum*. Two species belonging to the genus *Stephanocystis* are known to contain high levels of inorganic arsenic (Table 5). Recently, taxonomic DNA analysis of the family Sargassaceae by Draisma et al. (2010) found that the phylogenetic distance between *Sargassum* including *hijiki* and *Stephanocystis* is quite large. Therefore, all seaweeds belonging to the family Sargassaceae must be assessed for safety.

5. Conclusions

We report here that 7 weeks' consumption of a 3% *hijiki* diet resulted in an elevated body temperature, arsenic accumulation in blood and tissues, and various abnormal blood chemistries in rats. These results support further investigations to identify the health hazards related to the consumption of *hijiki* and other *Sargassum* species in humans.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

We express our sincere gratitude to Dr. Osamu Hayaishi, Chairman of the Board of Trustees and Director Emeritus at the Osaka Bioscience Institute (Osaka, Japan) for critical discussion and appraisal. We are grateful for the technical assistance by Ms. Hitomi Shizuka and Ms. Miki Otagi.

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