RISK ASSESSMENT OF THE NEGATIVE IMPACT ON HUMAN HEALTH OF ELEMENTAL CONTAMINANTS IN BROWN ALGAE (Laminariaceae) AND PRODUCTS BASED ON THEM

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Abstract

The content of elements Al, As (inorganic form), Cd, Cr, Co, Cu, Fe, Hg, I, Mn, Mo, Ni, Se, Pb, Sr, V, Zn in Laminariae thalli was measured by inductively coupled plasma mass spectrometry. Based on obtained data, the non-carcinogenic risks of the analyzed elements on human health where assessed when they enter the body together with a therapeutic dose of medicinal native products based on Laminariae thalli. It was found that the total hazard index at the 95th percentile concentrations level is 1.44. The maximum contribution to the hazard index is made attributed to iodine ($HQ_{95\%}$=1.37). The daily consumption of food products based on Laminariae thalli at a quantity of 5 g resulted in a hazard coefficient for iodine at various concentration levels is HQ_{med} = 0.98; $HQ_{95\%}$ = 5.86. The content of iodine in dietary supplements based on Laminariae thalli is close to the permissible limit value, where there is no risk to human health (3500 mg/kg).

Keywords: laminariae thalli, elemental contaminants, hazard coefficient, iodism, risk-based strategy, inductively coupled plasma mass spectrometry

I. Introduction

Currently, in the field of drug quality control, there is a tendency to move from the principle of harmlessness to the acceptance a negligible risk of negative effects caused by impurities entering the body along with the daily therapeutic dose of the drug. [1,2]. Within the framework of a risk-based strategy for controlling the content of elemental contaminants in drugs the actual concentration of an element is compared with its permitted daily concentration which depends on the therapeutic dose of the consumed drug and the route of its entry into the body. Therefore, this allowable intake is individualized for each drug.

At present, the risk-based strategy for controlling the content of elemental contaminants is not applied to herbal medicinal products (HMPs). It is generally accepted that the contamination of HMPs decreases significantly during the processing of herbal row materials (HRMs), therefore elemental contaminants in HMPs are not likely to cause substantial harm to human health. However, there is a separate category of HMPs whose level of contamination is equivalent to that of the original HRMs – these are native products based in HRM. Within the conceptual framework of the Eurasian Economic Union (EAEU), this term denotes preparations based on HRMs that do not contain excipients. Native products based on HRMs are used in medicine as pharmaceutical substances for production of other HMPs and directly as independent medicinal products. The latter are of particular interest, as all contaminants contained in them are completely transferred to the human body. The comparability of the contamination levels in native products based on

HRMs and origin HRMs underscores the relevance of implementing a risk-based quality control strategy for this category of HMPs.

Among the native products based on HRMs which are used as standalone HMPs, Laminariae thalli occupy a special place. They have laxative [3], antidiabetic [4], hepatoprotective [5], antioxidant [6], anti-inflammatory [7], bactericidal [8] and anticancer [9] effects, and are also used for the prevention and treatment of iodine deficiency diseases [10, 11]. Since Laminariae thalli are the main natural source of iodine for humans [12] and contain a large amount of beneficial bioactive substances [13], they are also used as a food product and a dietary supplement.

When assessing the risks of negative impact of elemental contaminants that enter the human body along with therapeutic dose of a medical preparation, the influence of heavy metals and arsenic is typically taken into account. In the case of Laminariae thalli, it is advisable to additionally include aluminum and iodine in the list of analyzed elements. Aluminum has toxic properties similar to those of heavy metals [14]. Excess iodine is also harmful to the human body, just like its deficiency [15]. The aim of this study is to evaluate the risk of negative impact on humans from heavy metals, arsenic, aluminum and iodine that enter the body along with medicinal native products, dietary supplements, and food product based on Laminariae thalli.

II. Methods

As objects of study, we used samples of self-collection thalli of pharmacopoeial species of Laminaria japonica (Aresch.), collected in the waters of Peter the Great Bay in the Pacific Ocean, and Laminaria saccharina (L.), collected in the waters of Solovetsky Island in the White Sea; samples of the phytopreparations and dietary supplements based on «Laminariae thalli (seaweed)» were purchased from pharmacies and supermarkets in Moscow. The self-collected samples were obtained in August 2020, dried in the sun for two days, and the species identification of the samples was confirmed through the use of macro- and microscopic analysis. Additionally, literature data on the content of elemental contaminants in the thalli of pharmacopoeial Laminariae species from different collection sites were also used [16].

The risks of exposure to the elements Al, As (inorganic form), Cd, Cr, Co, Cu, Fe, Hg, I, Mn, Mo, Ni, Se, Pb, Sr, V, Zn were assessed. The selective determination of inorganic forms of arsenic (iAs) in Laminariae thalli was conducted our own developed methodology based on solid phase extraction and inductively coupled plasma mass spectrometry (ICP-MS) [17]. Elemental analysis was performed an Agilent 7900 mass spectrometer with inductively coupled plasma. The quantitative determination of heavy metals and aluminum content was carried out according to our own developed and validated method [18], iodine - according to the method [19]. Intensity values were recorded for atomic mass units (AMU) as follows: Al - 27 AMU, V - 51 AMU, Cr - 52 AMU, Mn - 55 AMU, Fe - 56 AMU, Co - 59 AMU, Ni - 60 AMU, Cu - 63 AMU, Zn - 66 AMU, As - 75 AMU, Sr - 88 AMU, Mo - 95 AMU, Cd - 111 AMU, Hg - 202 AMU, Pb – 208 AMU, Se - 78 AMU, I - 127 AMU. To calculate the concentrations of the analyzed elements in the tested samples of Laminariae thalli, the calibration curve method was used. Three parallel test solutions were prepared for each sample. The final concentration values were determined as the arithmetic mean of the measured values.

III. Results

The main indicator of expected population morbidity growth due to the toxic properties of foreign chemicals in the studies objects of the environment is the non-carcinogenic risk. When assessing non-carcinogenic risk, it is assumed that there is a threshold below which harmful effects do not occur. The quantitative characteristic of non-carcinogenic risk is the hazard coefficient (HQ), which represents the ratio of the average daily intake of an elemental impurity (ADD) to its safe (reference) level of exposure (R_fD) [1]:

$$HQ = \frac{ADD}{RfD} \quad (1)$$

The ADD value was estimated using formula 2, which was proposed for environmental contaminants [20]:

$$ADD = \frac{C \bullet IR \bullet EF \bullet ED}{BW \bullet AT} \quad (2)$$

where C - the concentration of the investigated elemental impurity in Laminariae thalli, mg/kg;
IR - the consumption rate of Laminariae thalli, kg/day;
EF – the exposure frequency, days/year;
ED – the exposure duration, years;
BW – the average body weight of a human (70 kg);
AT – the averaging time of exposure, days.

In the calculating, the values of HQ for the analyzed elements in the Laminariae thalli and native products, the values of IR, EF were determined based on the maximum therapeutic dose of the phytopreparation and the course of its intake, and ED and AT were determined from the average life expectancy (70 years) and the age of starting the treatment. (from 12 years old): IR=0.003 kg; EF=30 days, ED=58 years, AT=365•ED. Two concentration levels (median and 95th percentile) of the elemental contaminant in the sample (C_{med} and $C_{95\%}$) were used. The primary data used to determine the values of C_{med} and $C_{95\%}$ are presented in our publication [16, 21]. The reference values (R_fD) for analyzed elements are provided in the integrated risk information system EPA [22]. The calculated values of ADD and HQ for heavy metals, arsenic, aluminum, iodine during the maximum course of treatment with the maximum therapeutic dose of native products based on Laminariae thalli are presented in Table. 1.

Table 1: *Hazard coefficients of elemental contaminants in native products based on Laminariae thalli*

Element	Sample size	RfD mg/kg•day	C_{med}($C_{95\%}$) mg/kg	ADD_{med} •10^{-3} ($ADD_{95\%}$ •10^{-3}), mg/kg•day	HQ_{med} ($HQ_{95\%}$)
Al	14	1,0	25,47 (334,3)	0,090 (1,177)	0,0001 (0,001)
iAs	14	0,0003	0,297 (2,308)	0,001 (0,008)	0,003 (0,027)
Cd	30	0,001[1]	0,595 (2,654)	0,002 (0,009)	0,002 (0,009)
Cr	18	1,5[2]	0,600 (1,744)	0,002 (0,006)	0,001•10^{-3} (0,004•10^{-3})
Co	16	0,0003	0,085 (0,405)	0,0003 (0,001)	0,001 (0,005)
Cu	31	0,04	1,392 (3,735)	0,005 (0,013)	0,0001 (0,0003)
Fe	33	0,70	116,0 (537,5)	0,409 (1,893)	0,003 (0,014)
Hg	19	0,0003[3]	0,023 (0,229)	0,0001 (0,001)	0,0003 (0,003)
I	17	0,01	1473 (3880)	5,191 (13,67)	0,519 (1,367)
Mn	30	0,14[1]	5,984 (20,41)	0,021 (0,072)	0,0002 (0,001)
Mo	7	0,005	0,948 (2,027)	0,003 (0,007)	0,0007 (0,001)
Ni	20	0,02[3]	0,470 (2,400)	0,002 (0,008)	0,0001 (0,0004)
Pb	31	0,0035	0,340 (4,550)	0,001 (0,016)	0,0003 (0,005)
Se	14	0,005	0,140 (3,120)	0,0005 (0,011)	0,0001 (0,002)
Sr	9	0,60	349,8 (867,7)	1,232 (3,056)	0,002 (0,005)
V	13	0,005	0,900 (2,177)	0,003 (0,008)	0,001 (0,002)
Zn	36	0,30	15,63 (42,88)	0,055 (0,151)	0,0002 (0,001)

[1]RfD value is for dietary supplements;
[2]RfD value is given for Cr (3+);
[3]RfD value is given for salts Hg, Ni

From the data in Table. 2 it can be observed that the values of the iodine HQ exceed the HQ values of elements Al, iAs, Cd, Cr, Co, Cu, Fe, Hg, Mn, Mo, Ni, Se, Pb, Sr, V and Zn by a thousand

or more times. Therefore, for food products and dietary supplements based on Laminariae thalli, we focused only on assessing the non-carcinogenic risk from the negative effects of iodine. The concentrations of iodine in the analyzed samples of food and dietary supplements based on Laminariae thalli are presented in Table. 2.

Table 2: *The content of iodine in food products and dietary supplements based on Laminariae thalli*

№	Sample	Concentration I, mg/kg	№	Sample	Concentration I, mg/kg
1	Seaweed (Laminaria japonica) natural shredded dried produced by RPG «Binom» LLC, Russia	1024	**7**	Laminaria seaweed, «Krevetka» LLC, Russia	126
2	"Belomorskaya zhemchuzhina (seaweed), Trade House " Sila prirody " LLC, Russia	350	**8**	Dried Laminaria, «SANATA» LLC, Russia	515
3	Laminaria, sun-dried seaweed, «Pozdnij zavtrak lajf» LLC, Russia	3563	**9**	Ground kelp for cocktails (powder), Las-Flores group LLC, Russia	300
4	Seaweed dried Laminaria, Individual Entrepreneur Boko O.V., Russia	2217	**10**	Dietary supplement Laminaria thalli (“Laminaria (seaweed)”, «Evalar» CJSC, Russia	3101
5	Algae White Sea food Laminaria, shredded, «Arhangel'skij vodoroslevyj kombinat» LLC, Russia	379	**11**	Dietary supplement Laminaria SUPERFOOD «Kron» LLC, Russia	3399
6	Dried seaweed Sakhalin VkusVill, «Mir Vodoroslej» LLC, Russia	287	**-**	-	-

When calculating the HQ value for iodine entering the human body through the consumption of food products based on Laminariae thalli (samples 1-9 from Table 2, C_{med} = 379 mg/kg, $C_{95\%}$ = 3024 mg/kg), the IR value was determined on the basis of literature data on the average daily consumption of brown algae by Europeans (5 g/day [23,24]). The option considered was the daily intake of Laminariae thalli throughout one´s lifetime, starting from the age of 12 (the age at which the consumption of native products based on Laminariae thalli begins). Under these conditions, the values obtained were ADD_{med} = 0.027; $ADD_{95\%}$ = 0.216; HQ_{med} = 2.71; $HQ_{95\%}$ = 21.60.

When calculating the HQ for iodine entering the human body through the consumption of dietary supplements based on Laminariae thalli, the guidelines provided by the manufacturers were followed. The recommended age for starting the intake of the dietary supplements is 18 years, with tablets of 200mg to be taken once daily ((IR = 0.0002, EF = 360 days , ED=52 years, AT=365•ED). Considering the declared concentration of iodine in the dietary supplements based on Laminariae thalli (0,1% per tablet), the calculated value are ADD=0.003 mg/(kg•day) and HQ=0.28.

IV. Discussion

The conclusion regarding the significance of the risk of the total negative effects of elemental contaminants on the human body is made based on the hazard index (HI). HI represents the sum of the HQ values of the analyzed elements at the median and 95th percentile levels (HI_{med} and HI_{95}). It is generally accepted that when the value of HI_{med} exceed 1, it indicates an unacceptable impact of elemental contaminants on human health requiring appropriate safety measures to be taken. The combination of HI_{med}<1 and $HI_{95\%}$<1 indicates no risk to human health from the contaminants. In situations where HI_{med}<1, but HI_{95}%>1, it is necessary to strengthen the control over the contaminants with the highest contribution to exposure.

For native products based on Laminariae thalli HI_{med} = 0.53 and $HI_{95\%}$ = 1.44. Therefore, it is necessary to strengthen the control over the iodine content in this group of HMPs, as it contributes the most to the total hazard index (HI). As a safety measure for consuming such herbal medicinal products, it is recommended to introduce an upper limit for iodine content. Currently, the State Pharmacopoeia of the Russian Federation only regulates the lower limit of iodine content (not less than 0,1%) [25]. It should be noted that the European Pharmacopoeia, unlike the Domestic Pharmacopoeia, the range of iodine content in Fucus algae is regulated (0,03 – 0,2%) [26]. The content of iodine in Fucus algae is significantly lower than in Laminariae algae. However, the regulated range of iodine content in Laminariae thalli recommended by the FDA (0,1-0,5% [27]) aligns well with the actual content of this element in pharmacopoeial species of Laminariae thalli (Laminaria saccharina (L.) and Laminaria japonica (Aresch.). It is recommended to include this range of iodine content in the State Pharmacopoeia of the Russian Federation.

The content of iodine in food products based on Laminariae thalli comparable is to that in the raw material. However, increasing the dosage and frequency of intake of the food products compared to the medicinal preparation is the reason why the HQ_{med} (2,71) and $HQ_{95\%}$ (21,6) significantly exceed 1. Therefore, daily consumption throughout life, starting from the age of 12, in the amount of 5g, leads to a serious risk of iodism. The safe daily intake of Laminariae thalli is 2g (HQ_{med}=1.083).

Our experimental data on the content of iodine in dietary supplements based on Laminariae thalli (3101 and 3399 mg/kg, samples 10, 11 of Table 2) indicate that the actual concentration of this element significantly exceed the value stated by the manufacturers on the packaging (0.1 % or 1000 mg/kg). The content of iodine in these dietary supplements is comparable to its content in raw materials and medicinal native products. As a result, long-term intake of dietary supplements based on Laminariae thalli may pose a risk of iodine poisoning. With the maximum intake course specified by the dietary supplements manufacturers (IR= 0.0002, EF=360 days, ED=52 years, AT=365•ED), the HQ=1 at an iodine concentration of 200 mg per tablet should not exceed 3500 mg/kg. As can be seen from the data in Table. 2, the actual iodine content in the analyzed dietary supplements is close to the maximum allowable value, at which there is no risk to human health.

Thus, the risk assessment of the negative impact on human health of elemental contaminants ingested through the consumption of products based on Laminariae thalli has shown that iodine makes the maximum contribution to exposure. It is concluded that there is a need to strengthen control over the iodine content in medicinal native products, dietary supplements and food products based on Laminariae thalli.

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