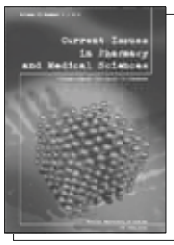

2012 Curr. Issues Pharm. Med. Sci. Vol. 25, No. 1

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy

Determination of the level of chromium and nickel in selected dietary supplements stimulating the immune system of the human body

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ABSTRACT

An important group of dietary supplements are those preparations stimulating the defence mechanism of the human body. Often powdered plant raw materials or dry extracts from herbs are included in these dietary supplements, therefore these preparations, apart from being biologically active, can contain considerable quantities of the mineral matter. The purpose of our work was to assess the levels of chromium and nickel in selected dietary supplemental products produced in the form of capsules and pills (*Acerola Plus*, *Cat's Claw*, *Maca*, *Maca 50 Plus*, *Spirulina*, *Spirulina Hawaian*, *Spirulina Pacifica*, *Vilcacora*, *Żeń-szeń* from the "Herbalist's Plants of 'Herbapol' in Cracow", *Żeń-szeń – Ginseng* from "KRKA", *Żeń-szeń* supplied by Naturell, *Żeń-szeń Korean* from "Walmark", *Żeń-szeń* supplied by "*Vita-complex*") and in liquid preparations (*Aloe* - juice, *Aloe vera* drinking gel, *Aloes Activ, Aloes Young*, *Bodymax Tonik*, *Ginsengin 400*, *Ginsenol*, *Noni* - juice, *Noni-vita*, *Noni Plus*). Prepared samples (3-5) of each of preparations were examined, in two parallel runs with analysis was carried out using a Pye Unicam SP 192 atomic absorption spectrometer, while applying specific determination parameters for given element. In supplements in the form of capsules and pills, the chromium content fluctuated, on average, from 0.33 μg/g (*Acerola Plus*), to 13.79 μg/g (*Spirulina*), and of nickel, from 0.16 μg/g (*Acerola Plus*), to 4.10 μg/g (*Żeń-szeń* (ginseng) – "Herbalist's Plants of 'Herbapol' in Cracow'). In the liquid dietary supplements that were tested, the values were as follows: the amounts of chromium were up to 0.12 μg/ml, while nickel was up to 0.27 μg/ml. In studied preparations, chromium and nickel levels diverged widely, depending on the form and composition of the dietary supplement. What is more, special predispositions of certain raw plant materials (marine alga – spirulina and of root of ginseng) were observed, for the accumulation of the determined micro-elements.

Keywords: dietary supplements, chromium, nickel, atomic absorption spectrometry

INTRODUCTION

Dietary supplements play important roles in engendering profitability in the world's pharmaceutical businesses. In 2008, the value of the market for dietary supplements in Poland alone, amounted to 21% of the daily income within the typical pharmacy. On a yearly basis, this amounted to over 1.1 bn PLN within the industry as a whole. This market-share is growing, since every year, new supplements are being registered. What is more, from the total number of supplements reported to the governing bodies of the industry, in the years 2000–2005, the majority of this market was based upon preparations of raw herbal materials [14, 17].

The growing demand for dietary supplements results from an increased interest in living healthy lifestyles. These preparations are taken by both young people and the elderly. A dietary supplement, as the term suggests, is something added to the everyday diet that provides scarce substances considered essential (or perceived to be essential) for the correct functioning of the human body. These include nutrients, vitamins and minerals or other substances demonstrating physiological action. Such items can be applied prophylactically or as prescribed supplements in the therapy for different disease entities [12, 17].

Preparations stimulating the immune system of the human body constitute an important group of these supplements. These preparations usually contain ingredients of plant origin which are imported as raw materials from Asia and South America [10, 15]. Among the raw ingredients in such studied dietary supplements which owe their immuno-stimulant properties to plant elements are: aloe, globefish, vilcacora, root poppies and ginseng, the marine alga spirulina, as well as acerola and noni [6, 10, 15].

Some plant raw materials contain considerable quantities of mineral compounds. This is because some plants can selectively accumulate certain elements. For example, experimental research reveals that that marine alga *Spirulina platensis* absorbs from a polluted environment, among other minerals: selenium, zinc, copper, manganese, iron and chromium (III). It then binds these elements into organic complexes [6]. It should be noted that micro-bioelements such as chromium and nickel are indispensable components of healthy living, but only in specific (trace) amounts, as excessive quantities of these micro-elements induce adverse action in the human body [1, 9].

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In relation to the rise of the quantity of supplements in the commercial market and the increased demand for these preparations, regulating bodies must ensure that they are safe for consumption and not a hazard for health. For this purpose, tests of level of chromium and nickel were performed in selected supplements whose ingredients display an influence on the human immunological defence mechanism.

MATERIALS AND METHODS

The studied dietary supplements were purchased in pharmacies in Lublin in 2010 (Table 1 and 2). Samples (3-5) of each preparation were examined, in two parallel runs. Every sample constituted a separate series of the production. To ensure accurate weight of mineralization, supplements in the form of pills were crushed in a mortar, while gelatin capsules were weighed in one piece, and liquid supplements, after thorough measuring out, were dried within quartz evaporating basins. Samples were then mineralized "dry" at the temperature of 450°C. The process of this mineralization was sped up using a water solution of nitric acid (V) of a concentration of about 20% (HNO_3 Suprapur, Merck). The ashes were then dissolved in water containing a 15% solution of hydrochloric acid (HCl Suprapur, Merck).

Table 1. Name, producer and composition of dietary supplements (in the form of capsules and tablets) stimulating the human immune system

No	Preparation name	Main components
1	Acerola Plus (tabl.); Puritan's Pride Inc., USA	Powdered acerola fruit, powdered grains of the buckwheat, buds of the wild rose (*Rosa canina*), citrus biflavonoids, extract from green pepper, extract from blackcurrant, rutin, hesperidine
2	Cat's Claw (caps.); Now Foods, USA	Powdered cat's claw (*Uncaria tomentosa*)
3	Maca (caps.); A-Z Medica, Sp. z o.o., Poland	Powdered maca root (*Lepidium meyenii* Walp.)
4	Maca 50 Plus(caps.); A-Z Medica, Sp. z o.o., Poland	Powdered maca root (*Lepidium meyenii* Walp.), antioxidative prefix: beta-carotene, vitamins – E, C; shark's cartilage
5	Spirulina (tabl.); Walmark, Sp. z o.o., Czech Rep.	Sea algae (*Spirulina platensis*), Vitamins: B_1, B_2, B_6
6	Spirulina Hawaian (caps.); Organic by Nature Inc., USA	Sea algae (*Spirulina platensis*) 100%
7	Spirulina Pacyfica (tabl.); Cyanotech Corp., USA	Sea algae (*Spirulina platensis*)
8	Vilcacora (caps.); Andean Medicine Centre Ltd., United Kingdom	Vilcacora (*Uncaria tomentosa*)
9	Żeń-szeń (ginseng), (caps.); Herbapol Kraków, Poland	Powdered ginseng root (*Ginseng radix*), iron oxides as capsule components
10	Żeń-szeń Ginseng, (caps.); KRKA, Slovenia	Dry extract from ginseng root (*Panax ginseng radicis extractym siccum*), iron oxides as capsule components
11	Żeń-szeń Ginseng (tabl.); Naturell AB, Sweden	Standardized extract from white Korean ginseng root (*Panax ginseng* C.A. Meyer)
12	Żeń-szeń koreański (Korean ginseng), (caps.); Walmark, Sp. z o.o., Czech Rep.	Extract from white Korean ginseng root, vitamin E (D-alpha tocopherol)
13	Żeń-szeń vita-complex (ginseng), (caps.); Olimp Lab., Sp. z o.o., Poland	Korean ginseng (Korean *Panax ginseng extract*.), American ginseng (*Panax quinquefolium extract*)

The levels of chromium were determined directly from the mineralisate, and that of nickel, from the organic phase, by inducing this element into a comprehensive bond with the ammonium salt of dithiocarbamate of pyrrolidine (APDC), in an environment containing a citrate buffer at pH = 6.8 and then extracting the complex through the use of methyl isobutyl ketone (MIBK) [16]. Analysis was carried out in a Pye Unicam SP 192 atomic absorption spectrometer, applying specific determination parameters for each given element [13].

Table 2. Name, producer and composition of liquid dietary supplements stimulating human immune system

No	Preparation name	Main components
1	*Aloe vera* – aloe juice; Laboratoria Natury, Alter Medica, Olfam, Sp.z o.o., Poland	Aloe juice (*Aloe vera*)
2	*Aloe vera* drinking gel juice with pulp pieces – gel; Laborat. Natury, Sp. z o.o., Bio Medica, Poland	Aloe juice with pulp pieces (*Aloe vera*)
3	Aloes Activ – aloe juice with extract of ginseng; Alter Medica, Poland	Aloe juice (*Aloe barbadensis*), ginseng extract (*Acanthopanax senticosu*s Maxim)
4	Aloes Young + żurawina – aloe juice and cranberry juice with acerola fruit; Alter Medica, Poland	Aloe juice (*Aloe barbadensis* – 70%), cranberry juice (*Oxycoccus palustris* Hill syn. *Vaccinum oxycoccus* – 25%), acerola powdered fruit (M*alpighia gabra* – 5%)
5	Bodymax Tonik; Axellus A/S, Denamrk	Standardized extract from ginseng GGE (*Panax ginseng* C.A. Meyer), Standardized extract from Japanese gingko (*Ginkgo biloba*), vitamins: B_1, B_2, B_6, PP, pantothenic acid
6	Ginsenging 400 – extract from ginseng with honey; MEHECO No 18, Guangiming Zhong Jie, China	*Ginseng radix extractum spissum* 3:1 – 400 mg, royal jelly 300 mg in 1 ampoule – 10 ml
7	Ginsenol; Krakowskie Zakłady Zielarskie „Herbapol", Kraków, Poland	Ginseng root tincture 1:5 (*Ginseng radix*)
8	Noni - noni fruit juice; Alter Medica, Poland	Noni fruit juice (*Morinda citrifolia*)
9	Noni-vita – noni fruit juice; Laboratoria Natury, Sp. z o.o., Poland	100% Noni fruit juice (*Morinda citrifolia*)
10	Noni Plus – juice of noni and cranberry fruits; Alter Medica, Poland	Noni fruit juice (*Morinda citrifolia*), cranberry juice (*Oxycoccus palustris*)

In the same conditions, analysis was performed of the certified reference material. This is a blend of Polish herbs deemed "Mixed Polish Herbs" (INST-MPH- 2) [7]. Declared amounts of examined elements in this referential material amounted to: chromium – 1.69 ± 0.13 μg/g; and nickel – 1.57 ± of 0.16 μg/g. Determined amounts were: chromium – 1.63 ± 0.07 μg/g; and nickel – 1.51 ± 0.08 μg/g.

The results of our analysis were arranged in tables 3, 4 and 5, giving the arithmetic mean, the standard deviation and the content range (min. – max.) – for each preparation.

RESULTS AND DISCUSSION

Chromium (III) plays important metabolic functions in the human body, but so far, an EAR value (Estimated Average Requirement) or a UL value (Tolerable Upper Intake) have not been set. Only an Adequate Intake (AI) value have been determined. This, for young women (from 19 to 50 of year of age) is 25 μg/day, and for men in this age group – 35 μg/day of chromium. For older women and men (from 51 up to 70 years) the AI value is 20 μg/day and 30 μg/day of chromium respectively [8, 9].

In the studied dietary supplements, a larger quantity of chromium was indicated in preparations containing the marine alga spirulina (*Spirulina platensis*): maximum 13.79 µg/g (in the *Spirulina* preparation "Walmark"); 10.86 µg/g (*Spirulina Pacifica*) and 8.75 µg/g (*Spirulina Hawaian*). It was stated earlier that this alga demonstrates peculiar predispositions to the accumulation of chromium (III). The capsules and pills based on other than spirulina derived material used in this study contained, on average, from 0.33 µg of chromium in 1 g of preparation (*Acerola Plus*), to 4.99 µg of this element in 1 g of ginseng from "Herbalist's Plant, in Cracow". After converting into individual mass preparation, it is still noticeable that a greater amount of chromium appeared in pills and capsules with spirulina, on average from 5.25 µg to 8.97 µg. Hence, when supplementing daily intake with the *Spirulina* – based supplement "Walmark" at the maximum recommended daily dose, we can introduce 44.85 µg of chromium on average into the organism. This constitutes over 100% of the standard established as AI (level of the sufficient consumption) for this element [8, 9].

In regard to liquid supplements, chromium levels ranged, on average from 0.02 µg/ml (*Aloes Young*) to 0.12 µg/ml in *Aloe vera* – drinking gel. This at the maximum recommended dose of 6.0 µg, constituted per day, constituted 17% of the recommended norm for men and 24% for women [8, 9].

A literature search in science journals indicates that the level of chromium in dietary supplements and other preparations (in the form of capsules and pills) ranged widely. In dietary preparations, it was stated, on average, from 0.15 µg/g to 50.64 µg of this element can be found in 1 gram of these supplements (preparations with the intentional addition of chromium) [2]. In particular, for several products, values were from 0.12 µg/g to 22.93 µg/g in *Chitobon* – preparation with the addition of chromium [5]; in preparations made from phytoestrogens, from 0.12 µg/g to 4.57 µg/g [3]; and in supplements containing anti-oxidizing agents, from 0.03 µg/g (*Garlic forte*), to 6.85 µg/g (*Maxi Vision*) [4]. Leśniewicz et al. determined that in some preparations studied, much greater amounts of chromium were found – up to 63.1 µg/g [11].

Nickel has been relatively recently recognized as an element essential for man, but its recommended daily intake (RDA) so far has not be assessed, nor has its Adequate Intake (AI). Only a UL value has been calculated. This for an adult male amounts to 1 mg/day of soluble salts of nickel [9].

The content of nickel in the studied dietary supplements, as with chromium, was also widely ranged. Preparations in the form of capsules and pills contained, on average from 0.16 µg/g (*Acerola Plus*), to 4.10 µg/g in supplied ginseng roots (*Żeń-szeń* from "Cracow Herbalist's Plants"). What is more, considerable quantities of this element were found in preparations based upon the marine alga – *Spirulina platensis*. On average, this ranged from 2.55 µg/g, to 2.63 µg/g. In particular, the amount of nickel contained within capsules and pills derived from spirulina showed a maximum quantity of 1.68 µg in in the *Spirulina*-based pill supplied by "Walmark". Expressing this quantity as a maximum twenty-four hour dose shows that taking this supplement contributes 9.18 µg of nickel per day.

In the liquid supplements tested, the amount of nickel in soluble form averaged from 0.02 µg of nickel in 1 ml in *Aloe vera*, *Aloes Young*, *Bodymax Tonic* water, up to 0.27 µg/ml in the Noni-vita preparation. In regard to the juice of the noni fruit (from *Noni-vita*), on average, this dosage was 10.80 µg per day at the recommended rate of daily consumption.

Table 3. The chromium content in dietary supplements stimulating the human immune system, $\mu g \cdot g^{-1}$ and µg/caps. (tabl.) and intake of chromium with maximal of daily dose (µg)

No	Preparation name	Chromium (Cr) content ($\mu g \cdot g^{-1}$)	Chromium (Cr) content (µg/caps. (tabl.)	Mean intake of chromium with maximal of daily dose (µg of Cr)
		Arithmetic mean, standard deviation and content (min. – max.)		
1	Acerola Plus, (tabl.), n* = 5, m* = 0.92	0.33 ± 0.08 0.27 – 0.44	0.31 ± 0.07 0.25 – 0.41	0.31
2	Cat's Claw (caps.), n = 5, m = 0.60 g	0.87 ± 0.07 0.78 – 0.94	0.52 ± 0.04 0.47 – 0.56	3.12
3	Maca, (caps.), n = 5, m = 0.40 g	0.97 ± 0.05 0.90 – 1.00	0.39 ± 0.02 0.36 – 0.40	1.17
4	Maca 50 Plus, (caps.), n = 5, m = 0.60 g	1.74 ± 0.31 1.43 – 2.22	1.05 ± 0.18 0.88 – 1.33	2.10
5	Spirulina (Walmark), (tabl.), n = 5, m = 0.65 g	13.79 ± 3.19 10.07 – 16.80	8.97 ± 2.08 6.55 – 10.92	44.85
6	Spirulina Hawaian, (caps.), n = 5, m = 0.60 g	8.75 ± 0.67 7.91 – 9.54	5.25 ± 0.40 4.75 – 5.72	31.50
7	Spirulina Pacyfica, (tabl.), n = 5, m = 0.50 g	10.86 ± 0.97 9.96 – 12.23	5.43 ± 0.49 4.98 – 6.12	32.58
8	Vilcacora, (caps.), n = 5, m = 0.56 g	0.70 ± 0.17 0.50 – 0.90	0.39 ± 0.09 0.28 – 0.50	2.34
9	Żeń-szeń (ginseng – Herbapol Kraków), (caps.), n = 5, m = 0.30 g	4.99 ± 1.34 3.29 – 6.09	1.50 ± 0.40 0.99 – 1.83	6.00
10	Żeń-szeń Ginseng (KRKA), (caps.), n = 5, m = 0.46 g	0.72 ± 0.07 0.64 – 0.80	0.33 ± 0.04 0.29 – 0.37	0.33
11	Żeń-szeń Ginseng (Naturell), (tabl.), n = 5, m = 0 40 g	0.65 ± 0.09 0.57 – 0.76	0.26 ± 0.03 0.23 – 0.30	0.52
12	Żeń-szeń koreański (Korean ginseng – Walmark), (caps.), n = 5, m = 0.50 g	0.65 ± 0.16 0.45 – 0.82	0.32 ± 0.08 0.23 – 0.41	0.32
13	Żeń-szeń vita-complex, (ginseng – Olimp), (caps.), n = 5, m = 0.78 g	0.59 ± 0.21 0.47 – 0.90	0.46 ± 0.16 0.37 – 0.70	0.46

n* – number of examined series of production
m* – mean mass of capsule or tablet

Table 4. The nickel content in dietary supplements stimulating the human immune system $\mu g \cdot g^{-1}$ and µg/caps. (tabl.) and intake of nickel with maximal of daily dose (µg)

No	Preparation name	Nickel (Ni) content ($\mu g \cdot g^{-1}$)	Nickel (Ni) content (µg/caps. (tabl.)	Intake of nickel with maximal of daily dose (µg of Ni)
		Arithmetic mean, standard deviation and content (min. – max.)		
1	Acerola Plus, (tabl.), n* = 5, m* = 0.92	0.16 ± 0.01 0.15 – 0.18	0.15 ± 0.01 0.14 – 0.17	0.15
2	Cat's Claw (caps.), n = 5, m = 0.60 g	0.24 ± 0.03 0.22 – 0.28	0.15 ± 0.02 0.13 – 0.17	0.90
3	Maca, (caps.), n = 5, m = 0.40 g	0.80 ± 0.19 0.60 – 0.96	0.32 ± 0.08 0.24 – 0.38	0.97
4	Maca 50 Plus, (caps.), n = 5, m = 0.60 g	0.57 ± 0.09 0.44 – 0.67	0.34 ± 0.06 0.26 – 0.40	0.68
5	Spirulina (Walmark), (tabl.), n = 5, m = 0.65 g	2.58 ± 0.46 1.84 – 3.09	1.68 ± 0.30 1.20 – 2.01	8.40

6	Spirulina Hawaian, (caps.), n = 5, m = 0.60 g	2.55 ± 0.50 2.11 – 3.07	1.53 ± 0.30 1.27 – 1.84	9.18
7	Spirulina Pacyfica, (tabl.), n = 5, m = 0.50 g	2.63 ± 0.70 1.81 – 3.24	1.32 ± 0.35 0.91 – 1.62	7.92
8	Vilcacora, (caps.), n = 5, m = 0.56 g	0.24 ± 0.06 0.18 – 0.30	0.14 ± 0.04 0.10 – 0.17	0.84
9	Żeń-szeń (ginseng – Herbapol Kraków), (caps.), n = 5, m = 0.30 g	4.10 ± 1.28 2.74 – 5.40	1.23 ± 0.39 0.82 – 1.62	4.92
10	Żeń-szeń Ginseng (KRKA), (caps.), n = 5, m = 0.46 g	2.03 ± 0.20 1.77 – 2.24	0.93 ± 0.09 0.81 – 0.96	0.93
11	Żeń-szeń Ginseng (Naturell), (tabl.), n = 5, m = 0.40 g	1.43 ± 0.26 1.04 – 1.61	0.57 ± 0.10 0.42 – 0.64	1.14
12	Żeń-szeń koreański (Korean ginseng – Walmark), (caps.), n = 5, m = 0.50 g	0.84 ± 0.14 0.65 – 0.96	0.42 ± 0.07 0.33 – 0.48	0.42
13	Żeń-szeń vita-complex, (ginseng – (Olimp), (caps.), n = 5, m = 0.78 g	0.99 ± 0.07 0.90 – 1.04	0.77 ± 0.20 0.70 – 0.81	0.77

n* – number of examined series of production
m* – mean mass of capsule or tablet

Table 5. The chromium and nickel content in dietary supplements stimulating the human immune system, μg ml^{-1} ; intake of chromium and nickel with maximal of daily dose (μg)

No	Preparation name	Chromium (Cr) content ($\mu g \cdot ml^{-1}$)	Intake of Cr with maximal of daily dose (μg of Cr)	Nickel (Ni) content ($\mu g \cdot ml^{-1}$)	Intake of Ni with maximal of daily dose (μg of Ni)
		Arithmetic mean (M), standard deviation (SD) and content (min. – max.)			
1	Aloe vera – aloe juice, n* = 5, v* = 50 ml	0.04 ± 0.01 0.03 – 0.04	2.00	0.02 ± 0.01 0.01 – 0.03	1.00
2	Aloes – aloe juice with pulp pieces –gel, n = 5, v= 50 ml	0.12 ± 0.01 0.11 – 0.13	6.00	0.11 ± 0.01 0.10 – 0.12	5.50
3	Aloes Activ – aloe juice with extract of ginseng, n = 5, v = 50 ml	0.03 ± 0.01 0.02 – 0.03	1.00	0.03 ± 0.01 0.02 – 0.03	1.00
4	Aloes Young – aloe juice with cranberry n = 5, v = 60 ml	0.02 ± 0.01 0.02 – 0.03	1.20	0.02 ± 0.01 0.02 – 0.03	1.20
5	Bodymax Tonik, n = 5, v = 50 ml	0.04 ± 0.01 0.03 – 0.04	2.00	0.02 ± 0.01 0.01 – 0.02	1.00
6	Ginsenging 400 – extract of ginseng with honey, n = 3, v = 10 ml	0.08 ± 0.02 0.06 – 0.10	0.80	0.07 ± 0.01 0.06 – 0.08	0.70
7	Ginsenol, n = 3, v = 5 ml	0.03 ± 0.01 0.02 – 0.03	0.15	0.25 ± 0.05 0.20 – 0.30	1.50
8	Noni – juice of noni fruits, n = 5, v = 40 ml	0.06 ± 0.02 0.04 – 0.08	2.40	0.17 ± 0.05 0.13 – 0.23	6.80
9	Noni-vita – juice of noni fruits, n = 5, v = 40 ml	0.06 ± 0.01 0.05 – 0.08	2.40	0.27 ± 0.02 0.24 – 0.29	10.80
10	Noni Plus – juice of noni and cranberry fruits, n = 3, v = 40 ml	0.05 ± 0.01 0.04 – 0.06	2.00	0.10 ± 0.01 0.09 – 0.11	4.00

n* – number of examined series of production
m* – mean mass of capsule or tablet

In the research we conducted, dieting supplements contained on average, from 0.07 μg/g to 2.74 μg of nickel in 1 g of the *Slim Trio* preparation [2], while other preparations of similar action showed averages from 0.11 μg/g to 3.35 μg/g (*Teavera*) [5]. In regard to supplements derived from phytoestrogens, the nickel content averaged from 0.23 μg/g, to 1.56 μg of nickel in 1 gram *Fito-Fem Forte* [3]. As to supplements containing antioxidants, the amounts of nickel averaged from 0.03 μg/g (*Alitol*), to 1.98 μg/g (*Vision Maxi*) [4]. Leśniewicz et al. determined that some of the preparations they studied had much greater amounts of nickel – up to 32.3 μg/g [11].

CONCLUSIONS

Based upon our experimental results, the following conclusions can be made:

1. Determined levels of chromium and nickel in examined supplements varied widely; depending on form and composition of preparation.
2. Specific cumulative properties of chromium and nickel were evident in preparations based upon the marine alga (spirulina) and in the root of ginseng.
3. Amounts of chromium taken in at the recommended maximum twenty-four hour dose of the dietary supplement containing the alga spirulina provides over 100% of the daily demand of the organism.

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