

Study on Toxic Metal Levels in Food Supplements

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The objective of this study was to evaluate the chronic-toxic risks posed by individual metals via food supplements consumption by computing the hazard quotient (HQ), considering the average daily metal intake ADD ($\mu\text{g}/\text{kg}\cdot\text{day}$), daily intake reference dose RfD ($\mu\text{g}/\text{kg}\cdot\text{day}$) suggested by the European Food Safety Authority (EFSA), heavy metal concentration C in food supplements ($\mu\text{g}/\text{kg}$), food supplements rate IR ($\text{kg}/\text{person}\cdot\text{day}$), and the average body weight BW (kg). For this study we took into consideration that the food supplements analyzed are consumed daily at maximum dose. Samples were digested with concentrated nitric and hydrochloric acid and analysed for their inorganic elemental compositions by ICP/MS. The hazard index (HI) was used to estimate total chronic-toxic risks of multiple metals on the assumption of dose additivity. Cd hazard index is over one and it can be considered a great issue due to its chemical toxicology. The Cd hazard index is much greater in the case of animal and mineral food supplements than vegetal supplements. In the case of the other heavy metals analyzed (Cr, Cu, Zn, As, Hg and Pb) the hazard index is very low so their toxicological potential is negligible.

Keywords: food supplements, heavy metals, chronic-toxic risks, hazard quotient, hazard index, toxicological potential

Food dietary supplements as defined in a law adopted in a Congress of the United States in 1994, called Dietary Supplement Health and Education Act (DSHEA), cover a wide range of products that include vitamins and minerals, herbal products, amino acids, extracts of tissues and other compounds. According to (DSHEA), dietary supplements DS, include gel caps, pills, capsules and tablets. More than half of American adults said they consumed at least a supplement per month from the category of multivitamin / mineral [1] to increase vitality and prevention of chronic diseases [2-4]. The European Food Safety Authority (EFSA) defines food supplements as concentrated sources of nutrients or other substances with a nutritional or physiological effect, whose purpose is to supplement the normal diet.

In Europe it is known an extension of their use, a significant increase occurring in countries such as United Kingdom, Germany, Italy, Poland. between 2003-2010 [5, 6].

Weight gain and obesity are major concerns of specialists from around the world, especially from USA, the means used to achieve this goal being energy-restricted diets, exercise and metabolic changes [7].

The use of dietary supplements is necessary in all cases because low calorie diets (less than 800 or 500 calories per day) may also be limited in essential nutrients, exercise can alter electrolyte balance and metabolic changes imply appetite decreased and increased resting metabolism especially in the presence of some botanical types of dietary supplements.

Due to the increasing consumption of supplements each year, the range of age groups that use them, study of their composition, presence of components that are not mentioned on prospectus to accompany them, becomes extremely important.

Supplements contamination can occur accidentally or intentionally and may have several reasons: contaminants are present in raw material, in manufacturing process can occur a cross-contamination due to inadequate execution of operations or improper hygiene, transport, storage, packaging or an intentional contamination to increase efficiency and sale of supplement [5].

Aim of this study is the determination of contribution of heavy metals brought into the body from a daily intake of supplements of mineral or vegetal and animal origin. Heavy metals, whose presence is evidenced in water, soil, air, plants and other organisms are very important pollutants because of their toxicity, their accumulation and the ease that enter in the food chain [8-15]. Metals for which there is sufficient data to prove toxicity are: Hg, Pb, Sb, Cd, Zn, Cu, Sn, Ag, Au, Ni, Cr, Co, V, si Al. As you can see, in the category of heavy metals were included essential elements as Zn, Cu, Ni, Cr, Co, and V that become toxic at higher doses than the RDA. Practically, all of metal combinations are toxic chemicals over a certain dose specific to each element.

Experimental part

Materials and methods

Samples analyzed were purchased from Romanian market, totalling 20 sorts of supplements. All solutions were prepared with reagent grade chemicals and ultra-pure water ($18\text{ M}\Omega\text{ cm}$); nitric acid and hydrochloric acid were ultrapure from Sigma Aldrich.

Heavy metals determination

Determination of heavy metals in different samples was performed using a mass spectrometer with inductively coupled plasma (ICP-MS Agilent Technologies 7500 Series) and samples were digested with concentrated nitric and hydrochloric acid. The validity of the applied method was assessed by the analysis of standard reference materials according to SR EN ISO 14082:2003.

Concentration (C) of heavy metals in samples obtained is expressed in mg / g sample and is calculated from the formula:

$$C = a \cdot \frac{V}{m} \quad (1)$$

where:

- a - concentration value measured by the device, [ppb];
- V - volume of acid that dissolved sample [mL];
- m - mass of mineralized sample [g].

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ICP-MS determination procedure

ICP-MS measurements were performed using a Agilent Technologies 7500 Series (USA). The samples solutions were pumped by a peristaltic pump from the samples. The ICP-MS parameters were: nebulizer 0.9 ml/min, RF power 1500 W, carrier gas 0.92 l/min, makeup gas 0.17L/min, mass range 7-205 uma, integration time 0.1 s, acquisition time 22.76 s. Detector parameters: discriminator 8 mV, analog HV 1770 V and pulse HV 1070 V.

Health risk assessment

Deterministic estimation of health risks

In this study we assessed the human health risks posed by chronic exposure to the heavy metals contained in food supplements. The chronic-toxic risks posed by individual metals via food supplements consumption was estimated computing the hazard quotient (HQ) by the following equations [16]:

$$HQ = \frac{ADD}{RfD} \quad (2)$$

$$ADD = \frac{C \cdot IR}{BW} \quad (3)$$

where ADD is the average daily metal intake ($\mu\text{g}/\text{kg}\cdot\text{day}$), RfD is the daily intake reference dose ($\mu\text{g}/\text{kg}\cdot\text{day}$) suggested by the European Food Safety Authority (EFSA), C is the mean heavy metal concentration in food supplements ($\mu\text{g}/\text{kg}$), IR is the food supplements rate ($\text{kg}/\text{person}\cdot\text{day}$), and BW is the average body weight (kg).

The hazard index (HI) was used to estimate total chronic-toxic risks of multiple metals on the assumption of dose additivity [16,17]:

$$HI = HQ_1 + HQ_2 + \dots + HQ_n \quad (4)$$

Dose additivity usually requires that all components act by the same mechanism. However, the HI is also commonly used to screen for components with the same critical target without regard to the mechanism, and even for components with different target organs. HI calculation does not consider the interactions among the mixture

components. Thus, the health hazard may be underestimated if the interactions are greater than additive, or be overestimated if the interactions are less than additives [17].

Results and discussions

Heavy metal concentrations in different food supplements

In this study have been analyzed 20 samples, presented in Romanian market, of different origins: animal origin [6], vegetal origin [12] and mineral origin [2]. In table 1 are presented the type of sample, intake, intake dose and origin of all the 20 samples analyzed.

The heavy metals levels of food supplements analyzed have been compared to the maximum allowable levels of them. The maximum allowable levels have been taken from the European legislation (EC 1881/2006 regulation) for Cd, Hg and Pb and from the USP Advisory Panel in Inorganic Impurities and Heavy Metals (USP 2008) for Cr, Zn and As. For Cu, a maximum allowable level in food supplements have not been established at European level, however the European Copper Institute recommends a maximum level of 5 mg/kg Cu in foodstuffs.

Sixteen food supplements from the Romanian market contained heavy metals concentrations greater than the maximum allowable levels (1 sample over passed the maximum allowable level of Cr and 14 of Cd respectively). The content of Cr of S-M-11 was higher with 5480% than the maximum allowable level. Even if the Cr is thought to be an essential element, the high contamination of its valent form can have a negative influence on the human body [18]. However the high content of Cr in this type of food supplement could not be taken as a contaminant, because Cr is an active compound of the food supplement analyzed. The level of Cr in the food supplements have been in the same range with those found in the case of the food supplements from the Croatian market [19]. The maximum allowable level of Cu has been over passed in two food supplements: in S-M-15 and S-V-18.

In the case of Zn, As, Hg and Pb neither a sample have over passed the maximum allowable level. However, in the case of Cd 14 of the 20 samples analyzed over passed

Sample code	Sample	Intake	Min.-Max. intake	Origin
S-A-1	Omega 6	Daily	3-6 capsules	animal
S-A-2	Omega 3	Daily	1-2 capsules	animal
S-A-3	Shark cartilage	Daily	2-4 capsules	animal
S-V-4	Silymarin	Daily	1-2 capsules	vegetal
S-A-5	Coral calcium	Daily	1-3 tablets	animal
S-A-6	Supplement for muscle weight	Daily	70-140 g	animal
S-V-7	Supplement for loss weight	Daily	25-50 g	vegetal
S-V-8	Vitamin C	Daily	1-2 capsules	vegetal
S-V-9	Lecithin	Daily	1-2 capsules	vegetal
S-A-10	Fish oil	Daily	2-3 capsules	animal
S-M-11	Cr forte	Daily	1 tablet	mineral
S-V-12	Spirulina	Daily	2-6 capsules	vegetal
S-V-13	Olive extract	Daily	1-2 tablets	vegetal
S-V-14	Food supplement for memory	Daily	1-2 capsules	vegetal
S-M-15	Multi vitamins	Daily	1 tablet	mineral
S-V-16	Protein bar	Daily	1-3 bars	vegetal
S-V-17	Papaya	Daily	1-2 tablets	vegetal
S-V-18	Propolis	Daily	4-6 capsules	vegetal
S-V-19	Alfalfa	Daily	4-5 tablets	vegetal
S-V-20	Q co-enzyme	Daily	2-4 capsules	vegetal

Table 1
FOOD SUPPLEMENTS ANALYZED
FROM THE ROMANIAN MARKET

Metal	Maximum allowable levels (mg/kg)	Metal levels (mg/kg)	> Mal
Cr	2.50	2.91 (0-54.81)	1
Cu	5 mg/kg	3.39 (0-30.96)	2
Zn	1500	31.80 (5-573.63)	0
As	1.5	0.14 (0-0.72)	0
Cd	seaweed 3 mg/kg other supplements 1 mg/kg	50.99 (0-500.10)	14
Hg	0.1 mg/kg	2.2·10 ⁻³ (0-0.0004)	0
Pb	3 mg/kg	0.00286 (0-0.0359)	0

Table 2
METAL LEVELS MEASURED IN 20 SAMPLES OF FOOD SUPPLEMENTS, EXPRESSED AS MEDIANS, WITH MINIMUM AND MAXIMUM VALUES GIVEN IN BRACKETS. > MAL REPRESENTS NUMBER OF SAMPLES ABOVE THE MAXIMUM ALLOWABLE LEVEL

the maximum allowable levels (according to the CE 1881/2006 regulation in the case of food supplements from seaweed the maximum allowable level is 3 mg/kg and in the other food supplements is 1 mg/kg). The food supplements with the highest concentration in Cd are represented by sea/ocean product. The Cd content from fish oil (S-A-10) over passed the maximum by 166 times, from S-M-11 over passed by 180 times, S-M-15 over passed by 83 times, S-A-3 by 98.3 times. In the case of the food supplements from sea/ocean products the high level of Cd could be as the results of the contaminated environment.

Human health risk assessment

Deterministic estimation of health risk

The estimated daily intake of heavy metals was compared with the daily intake reference dose recommended by the European Food Safety Authority to assess the potential health risk of heavy metals ingested with the food supplements consumption. For this study we took into consideration that the food supplements analyzed are consumed daily at maximum dose according to table 1. The risk of chronic-toxic effects, according to US EPA methods, is computed as the ratio of the dose resulting from exposure to site media to the maximum dose that is

believed to be safe (daily intake reference dose). This ratio is named hazard quotient (HQ). No significant risk of chronic-toxic effects exists when the hazard quotient ratio is less than one. If the HQ exceeds one, chronic-toxic effects may occur. The chronic-toxic effects tend to increase with increased HQ. On the other hand, the HI expresses the combined chronic-toxic effects of multiple metals.

The oral daily intake reference dose, established by the European Food Safety Authority for food and food supplements, were 2.85 µg/kg·day, 15.70 µg/kg·day, 142.86 µg/kg·day, 2 µg/kg·day, 0.35 µg/kg·day, 1.6 µg/kg·day and 3.5 µg/kg·day for Cr, Cu, Zn, As, Cd, Hg, and Pb respectively.

According to the data presented in table 3, Cr, Cu, Zn, As, Hg, Pb content in a daily intake of supplements for a 70 kg weight person do not over pass the EU regulations. However in the case of Cd content in food supplements 13 of the 20 samples analyzed over pass the maximum daily intake recommended by the EFSA (2012). The content of Cd in oil fish over passes the maximum daily intake by 35 times. In the figure 1, is presented the content of Cd in food supplements according to their nature.

	Cr µg/kg·day	Cu µg/kg·day	Zn µg/kg·day	As µg/kg·day	Cd µg/kg·day	Hg µg/kg·day	Pb µg/kg·day
S-A-1	0	0.042	0.171	0.002	3.196	0	0.001
S-A-2	0	0.013	0.009	0.002	0.225	0	0
S-A-3	0	0.007	0.083	0.022	4.824	0	0
S-V-4	0.029	0.008	0.001	0	0	0	0
S-A-5	0.002	0.001	0	0.023	1.374	0	0.001
S-A-6	0.015	0.099	0.504	0.005	1.828	0	0
S-V-7	0.004	0.105	2.072	0.002	1.723	0	0
S-V-8	0.004	0.036	0.117	0.001	0.737	0	0
S-V-9	0.001	0.005	0.022	0.001	0.322	0	0
S-A-10	0.002	0.104	0.403	0.003	12.315	0	0
S-M-11	0.284	0.029	0.074	0.001	0.935	0	0
S-V-12	0.006	0.072	0.055	0.006	1.162	0	0
S-V-13	0.002	0.025	0.006	0.005	0.445	0	0
S-V-14	0.008	0.048	0.008	0.001	0	0	0
S-M-15	0.018	0.562	10.404	0.006	1.519	0	0
S-V-16	0.014	0.345	5.024	0.005	7.313	7.63·10 ⁻⁵	0
S-V-17	0.002	0.014	0.015	0.001	0	0	0
S-V-18	0	0.456	0.248	0.019	0	0	0
S-V-19	0.017	0.067	0.091	0.004	0.090	1.45·10 ⁻⁵	0
S-V-20	0	0	0	0.001	0	0	0.001
S-A-1	2.85 ¹	15.70 ²	142.86 ²	2 ⁴	0.35 ³	1.6 ²	3.5 ⁴

Table 3
DAILY INTAKE OF FOOD SUPPLEMENTS

¹ EFSA 2012a, ² EFSA 2006, ³ EFSA 2012b, ⁴ DGHCP 2004 Directorate-General Health and Consumer Protection

² EFSA 2006 TOLERABLE UPPER INTAKE LEVELS FOR VITAMINS AND MINERALS

³ EFSA Journal 2012; 10(1):2551

⁴ Directorate-General Health and Consumer Protection - Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States, 2004

¹ EFSA, 2012a, EFSA Journal 2012; 10(10):2881, ² EFSA 2006, ³ EFSA 2012b, ⁴ DGHCP Directorate-General Health and Consumer Protection

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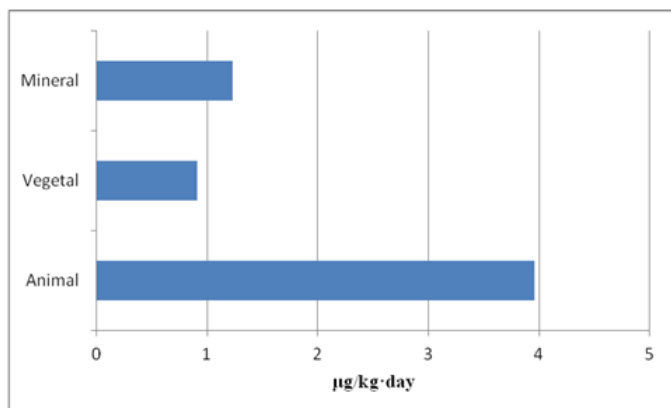


Fig. 1. Average daily intake of Cd in function of food supplements origin

Table 4
HAZARD INDEX OF HEAVY METALS PRESENTED IN FOOD SUPPLEMENTS ACCORDING TO THEIR ORIGIN

Food supplement type	Metal	HQ	Food supplement type	Metal	HQ	Food supplement type	Metal	HQ	HQ-media
Animal	Cr	0.001	Vegetal	Cr	0.003	Mineral	Cr	0.053	0.019
	Cu	0.003		Cu	0.006		Cu	0.019	0.009
	Zn	0.0013		Zn	0.004		Zn	0.037	0.014
	As	0.005		As	0.002		As	0.002	0.003
	Cd	11.31		Cd	2.81		Cd	3.55	5.890
	Hg	0		Hg	$4.73 \cdot 10^{-6}$		Hg	0	0
	Pb	$7 \cdot 10^{-3}$		Pb	$2.19 \cdot 10^{-3}$		Pb	0	0

It can be observed that the food supplements with a vegetal origin, the Cd content is the lowest (5 of the 12 vegetal samples are free of Cd), while the animal food supplements have the greatest content of Cd.

In table 4 is presented the hazard index of the heavy metals presented in food supplements according to their origin. It can be observed that Cd hazard index is over one and it can be considered a great issue due to its chemical toxicology. The Cd hazard index is much greater in the case of animal and mineral food supplements than vegetal supplements. In the case of the other heavy metals analyzed (Cr, Cu, Zn, As, Hg and Pb) the hazard index is very low so their toxicological potential is negligible.

Conclusions

Dietary supplements, food or nutritional supplements are free for sale, so that the dose intake daily by consumer, ranging from adolescents to the elderly is in its sole discretion, so that all calculations ingested daily dose were performed taking into account maximum amount provided in the prospectus of supplement. The analysis of heavy metal content of supplements analyzed was observed that only Cd is exceeded and the highest content being in supplements of animal origin (the content of Cd in oil fish over passes the maximum daily intake by 35 times). A consumption of several supplements in the day could cause drastic cadmium admitted content exceeding with serious health consequences. To protect public health, Food and Drug Administration (FDA) issued to producers of supplements guide of good practice (GMP) which offers certification and verification program for plant manufacturing and ingredients used. The rules of good practice (GMP) proposed the need to test raw materials for supplements to determine heavy metals, pesticides and industrial contaminants.

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Manuscript received: 12.10.2016