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Exposure to Metals from the Consumption of Energy Drinks - Dietary Intake Assessment

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Abstract

Energy drinks are frequently consumed by young people due to their stimulating properties. Taurine, vitamins, plant extracts as ginseng or guarana and minerals like Na, Mg, K, among others, are the main components. Considering the increasing consumption of these beverages, the objective of this study was to determine the content of metals (Na, K, Ca, Mg, Co, Cr, Cu, Fe, Mn, Mo, Zn, B, Ba, Li, Ni, V, Al, Sr, Cd, Pb) in 50 samples of energy drinks by ICP OES to assess the nutrition evaluation and the toxicological risk assessment derived from the consumption of energy drinks. Na (370 mg/L) was the most abundant macroelement, while Cu (3.64 mg/L) was the most remarkable trace element. Al (0.08 mg/L) was the most abundant toxic metal, followed by Pb (0.0005 mg/L) and Cd (0.0002 mg/L). A daily consumption of 250 mL of energy drinks contributes greatly to the dietary intake of the essential metals like Na with 6.17% of the recommended daily intake (RDI) and Fe with 3.64% of the RDI. The contribution percentages of toxic metals (Al, Cd, Pb) were under 4% of their respective reference values. In the case of acute consumers (750 mL), the contribution percentage to the RDI of Na was 18.5% and 11.9% of the TDI of Pb. Despite the presence of toxic metals like Al, Pb and Cd in these beverages the low levels detected do not pose a risk to consumer's health, maximum levels of metals such as Al, Cd and Pb in energy drinks should be incorporated into current legislation and monitored.

Keywords: *Energy Drinks; Metals; Nutritional Evaluation; Risk Assessment*

Abbreviations

EDI: Estimated Daily Intake; EFSA: European Food Safety Authority; ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry; LOQ: Limit of Quantification; NIST: National Institute of Standards and Technology; RDI: Recommended Daily Intake; SD: Standard Deviation; TDI: Tolerable Daily Intake; TWI: Tolerable Weekly Intake; UL: Upper Level Intake

Introduction

The energy drinks are a group of non-alcoholic carbonated beverages characterized by a stimulant effect [1]. The consumption of energy drinks has increased considerably in the last decades. The areas with the highest consumption per capita are North America, Asia/Pacific and the Western Europe [2].

These beverages contain mainly caffeine a stimulant that increases alertness and concentration (70 - 400 mg/L of caffeine) [2]. In addition, energy drinks contain taurine, vitamins, plant extracts as ginseng or guarana and minerals like Na, Mg, K, among others. The combination of these compounds confers to these drinks their stimulant properties [2-4]. Energy drinks are associated with and increased energy and vitality, improved performance (both physical and intellectual), reduced cognitive deficits and promoted wakefulness [4-6].

Energy drinks are popular among the young adult population and the rapid expansion of consumption of these beverages has been one of the most notable trends in the soft drinks market [2,7-9]. According to the EFSA (European Food Safety Authority) report about the consumption of energy drinks in Europe [2], the consumption of energy drinks is classified as mean chronic (125 mL/day), high chronic (350 mL/day) and acute (750 mL/day). The consumption patterns showed that 30% of European adults are consumers of energy drinks and among these around the 12% are high chronic consumers with an average consumption of 4.5L a month. Among the 68% of the European adolescents (10 - 18 years) that are energy drinks consumers, a 12% are high chronic consumers with an average consumption of 7L per month and a 12% are high acute consumers consuming at least 1.065L per single session [2].

Diet constitutes an important source of metals for humans [10-12]. The energy drinks contain minerals like macroelements (Na, K, Mg, Ca), which are essential to human and required daily or trace elements (Fe, Cu, Co, Cr, Zn, Mn, Mo), that are necessary to develop several physiological functions into the human organism [13,14]. However, these beverages can contain non-essential elements (Ni, Sr, V, Li, B, Ba), which are present in the environment and are essential to other animal and vegetal organisms and toxic metals (Al, Cd, Pb) [15]. Al is a neurotoxic agent whose high levels is brain are related to neurodegenerative diseases as Alzheimer [16]. Cd and Pb are persistent pollutants in the environment and known food contaminants, both metals cause several damages such as cardiovascular disease, osteoporosis, among other health effects [17-19].

The determination of metals in these drinks and the nutritional and toxicological assessments derived from the consumption of energy drinks is a novel field of research in food science. Therefore, the objectives of this study were to determine the metal content (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V, Zn) of energy drinks to assess the nutritional profile and toxicological risks according to the metal intake and their contributions to the reference values set by different institutions.

Material and Methods

Sampling

A total of 50 samples of different energy drinks presented in tin containers of a volume between 250 and 500 mL. were analyzed. The samples were acquired in different selling points of Tenerife (Canary Islands, Spain).

Sample treatment and analytical procedure

The degasification was performed by the previous treatment of the samples with ultrasound for 1 minute. Once degassed, 25 mL of the sample were placed in porcelain crucibles (Staalich, Germany) and dried in a stove (Nabertherm, Germany) at 80°C for 48h. The samples were subjected to acid digestion with HNO_3 65% (HoneyBell Fluka, Germany) and then, were introduced in a muffle furnace to the incineration process at 450°C for 24h. The obtained white ashes were dissolved in 1.5% HNO_3 in a total volume of 50 mL. The dissolution was transferred to PET containers to their analysis [18].

Metals were analyzed by ICP-OES (inductively coupled plasma optical emission spectrometry) using a Thermo Scientific iCAP 6000 spectrometer series (Walthan, USA). This reference method used for the determination of metals, is highly sensitive and presents an excellent reproducibility rate [19]. The instrumental conditions were as follow: gas flow (nebulizing gas flow, auxiliary gas flow) 0.5 L/min, approximately radiofrequency power 1150W; stabilization time 0s, pump flow when injecting the sample 50 rpm.

Table 1 shows the instrumental quantification limits and the wavelengths (nm) for each analyzed metal. Instrumental quantification limits in terms of reproducibility, were calculated as ten times the standard deviation (SD) resulting from analysis of 15 targets of acid digest [20].

Metal	Wavelength (nm)	Quantification Limit (LOQ) (mg/L)
Al	167.0	0.012
B	249.7	0.0012
Ba	455.4	0.0005
Ca	317.9	1.955
Cd	226.5	0.001
Co	228.6	0.002
Cr	267.7	0.008
Cu	327.3	0.012
Fe	259.9	0.009
K	769.9	1.884
Mg	279.1	0.013
Mn	257.6	0.008
Mo	202.0	0.002
Na	589.6	3.655
Ni	231.6	0.003
Pb	220.3	0.001
Sr	407.7	0.003
V	310.2	0.005
Zn	206.2	0.007

Table 1: *Instrumental quantification limits (mg/L) and wavelengths (nm) for each metal*

Quality controls were chosen by the criterion of method accuracy and this was established by the average recovery obtained when measuring a reference material under reproducible conditions. The reference material used was 1515 SRM Apple Leaves from the National Institute of Standards and Technology (NIST) (Gaithersburg, USA). In the case of Li, the quality controls were performed by additions of known amounts of this metal in water. Table 2 shows the recovery percentages which were adequate (over 96.5%) (Table 2).

Material	Metal	Obtained concentration (mg/kg)	Certified concentration (mg/kg)	Recovery (%)
SRM 1515 Apple Leaves	Al	286 ± 9	285.1 ± 26	99.7
	B	27.0 ± 2.0	27.0 ± 1.5	99.9
	Cr	0.30 ± 0.00	0.29 ± 0.03	97.8
	Mo	0.09 ± 0.01	0.09 ± 0.02	99.4
	Sr	25.0 ± 2.0	24.6 ± 4.0	98.3
SRM 1548a Typical Diet	Ba	1.10 ± 0.10	1.13 ± 0.09	102.5
	Ni	0.37 ± 0.02	0.38 ± 0.04	102.3
	Pb	0.044 ± 0.000	0.044 ± 0.013	98.9

SRM 1567a Wheat Flour	Ca	0.02 ± 0.00	0.02 ± 0.02	101.4
	Cd	0.026 ± 0.002	0.026 ± 0.008	98.4
	Co	0.006 ± 0.00	0.006 ± 0.002	102.4
	Cu	2.1 ± 0.2	2.09 ± 0.4	99.7
	Fe	14.1 ± 0.5	13.9 ± 0.3	98.9
	K	0.133 ± 0.003	0.132 ± 0.02	99.3
	Mg	0.04 ± 0.00	0.04 ± 0.03	102.6
	Mn	9.4 ± 0.9	9.6 ± 1.5	102.4
	Na	6.1 ± 0.8	6.1 ± 0.3	99.2
	V	0.011 ± 0.00	0.011 ± 0.00	99.4
	Zn	11.6 ± 0.4	11.9 ± 0.2	102.7

Table 2: *Certified concentration in mg/kg (mean ± SD, n = 3) of the reference materials and recovery study (%) of the analyzed metals.*

Statistical analysis

The statistical analysis was performed using the IBM Statistics SPSS 22.0 package for Mac®. Firstly, to study the normality of the results the Kolmogorov-Smirnov and Shapiro-Wilk tests were used. Then, it was study the homogeneity of variance the Levene test was used. Since the results did not follow a normal distribution, it was applied the Kruskal-Wallis non-parametric test and the Mann-Whitney U test [21-24] to establish whether there were significant differences between samples. Values of $p \leq 0.05$ were considered statistically significant.

Calculations for the intake assessment

In order to assess the nutritional profile and the toxic risk, is necessary to calculate the estimated daily intake (EDI), which is obtained as follow:

EDI (mg/day) = Mean energy drink consumption (L/day) · Metal concentration (mg/L)

Then, the EDI values are used to obtain the percentage of contribution to the recommended or maximum values, which is calculated:

Contribution (%) = [EDI (mg/day)/Limit value] · 100

Results and Discussion

Metals in energy drinks

Table 3 shows the maximum and minimum concentration recorded as the mean concentration (mg/L) and their standard deviations (SD) for each metal analyzed.

Among the macroelements, Na is the most abundant with levels between 61.8 - 696 mg/L. The remaining macroelements followed the sequence K > Ca > Mg. Statistical differences ($p < 0.05$) were found in the content of K, Mg, Ca and Na between the different analyzed energy drinks.

Metal	Parameter	Value	Reference	Mean concentration (mg/L)	EDI[b] (mg/day)	Contribution[c] (%)
Al	TWI	1 mg/kg bw/week	[27]	1.00	0.25	2.56
Cd		2.5 µg/kg bw/week	[28]	0.20[a]	0.00005	0.20
Sr	TDI	0.13 mg/kg bw/day	[29]	0.10	0.03	0.34
Ba		200 µg/kg bw/day	[30]	0.16	0.04	0.29
Ni		2.8 µg/kg bw/day	[31]	0.02	0.005	2.61
Pb		0.5 µg/kg bw/day	[32]	5.44[a]	0.0014	3.97
B	UL	17-20 mg/day	[33]	0.08	0.02	0.12
V		1.8 mg/day		0.03	0.008	0.44
Ca	RDI	900 - 1000 mg/day	[34]	40.4	10.1	1.12
Cr		35 mg/day (men) - 25 mg/day (women)		1.43[a]	0.0004	-
Cu		1.1 mg/day		0.16	0.04	3.64
Fe		9 mg/day (men) - 18 mg/day (women)		0.26	0.07	0.72 (men) - 0.36 (women)
K		3100 mg/day		134	33.4	1.08
Mg		350 mg/day (men) - 300 mg/day (women)		39.8	9.95	2.84 (men) - 3.34 (women)
Mn		2.3 mg/day (men) - 1.8 mg/day (women)		0.05	0.01	0.48 (men) - 0.61 (women)
Mo		45 mg/day		0.77[a]	0.19	0.43
Na		1500 mg/day		370	92.6	6.17
Zn		9.5 mg/day (men) - 7 mg/day (women)		0.14	0.04	0.42 (men) - 0.57 (women)

Table 4: *Estimated daily intake (EDIs) values and contribution percentage to the recommended daily intake (RDI) for normal consumers (250 mL/day)*

[a]*: Expressed in µg/L.*

[b]*: Daily energy drinks consumption of 250 mL/day (1 container per day).*

[c]*: Body weight of and adult set on 68.48 kg [35].*

[d]*: TWI, tolerable weekly intake; TDI, tolerable daily intake; UL, upper level intale; RDI, recommended daily intake.*

For trace elements, the order of sequencing based on the average concentration was Fe ~ Li > Ba > Cu > Zn > B > Mn > V > Ni > Cr > Co > Mo. Cr, Co and Mo mean concentration values were very low (1.43 mg/L, 0.85 mg/L and 0.77 mg/L, respectively). Statistical differences ($p < 0.05$) were found for Cr, Fe, Mn, Ni, Sr, V, Zn, Co and Mo among the analyzed energy drinks. Li (0.29 mg/L), Ba (0.16 mg/L) and B (0.08 mg/L) showed very low concentrations but as Olugbuyiro and Oseh (2011) reported, the migration of these metals from utensils and metal equipment to the beverage may happen [25].

With respect to the toxic metals, Al was the most abundant metal with concentrations between 0.20 and 3.69 mg/L. Pb concentrations were between < 0.001 - 0.04 mg/L and, Cd levels were between < 0.001 and 0.008 mg/L. The statistical analysis shows statistical differences

($p < 0.05$) in the Cd and Pb content between the analyzed energy drinks. The European legislation does not set a maximum allowed for Cd, Pb and Al in energy drinks [26]. It is strongly necessary to set regulations on the maximum limits for toxic metals in these products.

Dietary intake assessment

The table 4 shows the estimated daily intake (EDI) and the contribution percentages of the studied metals according to a normal energy drinks consumer who drinks 1 can/day of 250 mL [2]. This consumption contributes greatly to the recommended daily intakes (RDI) of Na (6.17% adults), Cu (3.64% adults) and Mg (2.84% men, 3.34% women). In addition, this consumption contributes with a 3.97% of the TDI for Pb and with 2.56% of the TWI for Al. However, the contribution percentages to the toxic metals' intake are under 4% of their respective reference values.

Metal	Parameter	Value	Reference	Mean concentration (mg/L)	EDI[b] (mg/day)	Contribution[c] (%)
Al	TWI	1 mg/kg bw/week	[27]	1.00	0.25	2.56
Cd		2.5 µg/kg bw/week	[28]	0.20[a]	0.00005	0.20
Sr	TDI	0.13 mg/kg bw/day	[29]	0.10	0.03	0.34
Ba		200 µg/kg bw/day	[30]	0.16	0.04	0.29
Ni		2.8 µg/kg bw/day	[31]	0.02	0.005	2.61
Pb		0.5 µg/kg bw/day	[32]	5.44[a]	0.0014	3.97
B	UL	17-20 mg/day	[33]	0.08	0.02	0.12
V		1.8 mg/day		0.03	0.008	0.44
Ca	RDI	900 - 1000 mg/day	[34]	40.4	10.1	1.12
Cr		35 mg/day (men) - 25 mg/day (women)		1.43[a]	0.0004	-
Cu		1.1 mg/day		0.16	0.04	3.64
Fe		9 mg/day (men) - 18 mg/day (women)		0.26	0.07	0.72 (men) - 0.36 (women)
K		3100 mg/day		134	33.4	1.08
Mg		350 mg/day (men) - 300 mg/day (women)		39.8	9.95	2.84 (men) - 3.34 (women)
Mn		2.3 mg/day (men) - 1.8 mg/day (women)		0.05	0.01	0.48 (men) - 0.61 (women)
Mo		45 mg/day		0.77[a]	0.19	0.43
Na		1500 mg/day		370	92.6	6.17
Zn		9.5 mg/day (men) - 7 mg/day (women)		0.14	0.04	0.42 (men) - 0.57 (women)

Table 4: *Estimated daily intake (EDIs) values and contribution percentage to the recommended daily intake (RDI) for normal consumers (250 mL/day)*

[a]: Expressed in µg/L.

[b]: Daily energy drinks consumption of 250 mL/day (1 container per day).

[c]: Body weight of and adult set on 68.48 kg [35].

[d]: TWI, tolerable weekly intake; TDI, tolerable daily intake; UL, upper level intale; RDI, recommended daily intake.

The table 5 shows the estimated daily intakes (EDIs) and the contribution percentages of the studied metals according to a high chronic consumer (350 mL of energy drinks/day). That consumption contributes to the daily requirements of macroelements like Na (8.67% adults) and Mg (3.97% men, 4.63% women), trace elements such as Cu (5.45% adults) and toxic metals, meaning a contribution percentage of 5.56% of the TDI for Pb and a contribution percentage of 3.58 of the TWI for Al. Also, is remarkably the contribution percentage of 3.65% of the TDI for Ni.

Metal	Parameter	Value	Reference	Mean concentration (mg/L)	EDI[b] (mg/day)	Contribution[c] (%)
Al	TWI	1 mg/kg bw/week	[27]	1.00	0.35	3.58
Cd		2.5 μg/kg bw/week	[28]	0.20[a]	0.00007	0.29
Sr	TDI	0.13 mg/kg bw/day	[29]	0.10	0.04	0.45
Ba		200 μg/kg bw/day	[30]	0.16	0.06	0.44
Ni		2.8 μg/kg bw/day	[31]	0.02	0.007	3.65
Pb		0.5 μg/kg bw/day	[32]	5.44[a]	0.002	5.56
B	UL	17-20 mg/day	[33]	0.08	0.03	0.18
V		1.8 mg/day		0.03	0.01	0.56
Ca	RDI	900 - 1000 mg/day	[34]	40.4	14.1	1.57
Cr		35 mg/day (men) - 25 mg/day (women)		1.43[a]	0.0005	-
Cu		1.1 mg/day		0.16	0.06	5.45
Fe		9 mg/day (men) - 18 mg/day (women)		0.26	0.09	1 (men) - 0.5 (women)
K		3100 mg/day		134	46.9	1.08
Mg		350 mg/day (men) - 300 mg/day (women)		39.8	13.9	3.97 (men) - 4.63 (women)
Mn		2.3 mg/day (men) - 1.8 mg/day (women)		0.05	0.02	0.87 (men) - 1.11 (women)
Mo		45 mg/day		0.77[a]	0.0003	-
Na		1500 mg/day		370	130	8.67
Zn		9.5 mg/day (men) - 7 mg/day (women)		0.14	0.05	0.53 (men) - 0.71 (women)

Table 5: *Estimated daily intake (EDIs) values and contribution percentage to the recommended daily intake (RDI) for high chronic consumers (350 mL/day).*

[a]: Expressed in μg/L.

[b]: Daily energy drinks consumption of 350 mL/day.

[c]: Body weight of and adult set on 68.48 kg [35].

[d]: TWI, tolerable weekly intake; TDI, tolerable daily intake; UL, upper level intale; RDI, recommended daily intake.

The table 6 shows the estimated daily intakes (EDIs) and the contribution percentages of the studied metals according to an acute consumer. The consumption of 750 mL per day of the analyzed energy drinks contributes significative to the recommended daily intake of Na (18.5% in adults), Mg (8.54% in men, 9.97% in women), Cu (10.9% in adults) and represents a 11.9% of the TDI of Pb and a 7.66% of the TWI of Al.

Metal	Parameter	Value	Reference	Mean concentration (mg/L)	EDI[b] (mg/day)	Contribution[c] (%)
Al	TWI	1 mg/kg bw/week	[27]	1.00	0.75	7.66
Cd		2.5 µg/kg bw/week	[28]	0.20[a]	0.002	0.61
Sr	TDI	0.13 mg/kg bw/day	[29]	0.10	0.08	0.90
Ba		200 µg/kg bw/day	[30]	0.16	0.12	0.88
Ni		2.8 µg/kg bw/day	[31]	0.02	0.02	10.4
Pb		0.5 µg/kg bw/day	[32]	5.44[a]	0.004	11.9
B	UL	17-20 mg/day	[33]	0.08	0.06	0.35
V		1.8 mg/day		0.03	0.02	1.11
Ca	RDI	900 - 1000 mg/day	[34]	40.4	30.3	3.37
Cr		35 mg/day (men) - 25 mg/day (women)		1.43[a]	0.001	0.003 (men) - 0.004 (women)
Cu		1.1 mg/day		0.16	0.12	10.9
Fe		9 mg/day (men) - 18 mg/day (women)		0.26	0.29	3.22 (men) - 1.61 (women)
K		3100 mg/day		134	101	3.26
Mg		350 mg/day (men) - 300 mg/day (women)		39.8	29.9	8.54 (men) - 9.97 (women)
Mn		2.3 mg/day (men) - 1.8 mg/day (women)		0.05	0.04	1.74 (men) - 2.22 (women)
Mo		45 mg/day		0.77[a]	0.0006	0.001
Na		1500 mg/day		370	278	18.5
Zn		9.5 mg/day (men) - 7 mg/day (women)		0.14	0.11	1.16 (men) - 1.57 (women)

Table 6: *Estimated daily intake (EDIs) values and contribution percentage to the recommended daily intake (RDI) for high acute consumers (750 mL/day).*

[a]: Expressed in µg/L.

[b]: Daily energy drinks consumption of 750 mL/day.

[c]: Body weight of and adult set on 68.48 kg [35].

[d]: TWI, tolerable weekly intake; TDI, tolerable daily intake; UL, upper level intale; RDI, recommended daily intake.

Instead of this fact, even in the case of the acute consumers, the metal content found in the analyzed energy drinks does not pose a health risk but should be considered a source of dietary metals.

Conclusions

The present study confirms that energy drinks are a source of essential elements, mainly Na, Mg and Cu. Despite the presence of toxic metals like Al, Pb and Cd in these beverages the low levels detected do not pose a risk to consumer's health, maximum levels of metals such as Al, Cd and Pb in energy drinks should be incorporated into current legislation and monitored.

The data resulting from this study demonstrate the need to assess metal concentrations in food to establish safe limits and overall intakes for the population. This study enriches current knowledge about the metal content in energy drinks, providing new data for assessing the quality and safety of these new foods.

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