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Frequency of consumption of coffee beverages in the city of Mostar and caffeine intake

Aiša Širbegović[1], Aida Šukalić[1]*, Maida Đapo-Lavić[2], Alma Mičijević[1], Alma Leto[1]

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Abstract

The aim of this study was to examine the frequency of consumption of coffee beverages in the city of Mostar. In 2019, an analysis of caffeine content was performed on HPLC in 10 different samples of coffee beverages.

Samples of coffee were taken from the market of the city of Mostar by random selection. In addition to the High-performance liquid chromatography (HPLC) method, the study was conducted using empirical and descriptive methods. An assessment of daily (EDI) and weekly intake (EWI) was also performed were on the base of determined values of caffeine content in 10 different coffee samples.

The acute toxic dose of caffeine is not well defined, but it is considered more than 10 grams of caffeine per day for adults, while in most countries it is not recommended that more than 450 mg of caffeine be consumed per day.

The samples were found to be in accordance with the EFSA Scientific Opinion (European Food Safety Authority) stating that a single dose of 200 mg of caffeine from all sources does not pose a risk to the health of healthy adults (EFSA, 2015).

Key words: Coffee, caffeine, acute toxic dose, risk.

Introduction

Coffee is a universal product, represents a special category of beverages and can be consumed on all occasions (Fujioka and Shibamoto, 2008; Ferraz et al., 2010; Martins and Gloria, 2010; Misik et al., 2010). Coffee has long been valued for its taste and, more importantly, stimulating effect. Given the tradition of drinking coffee in our society and the large number of different types of coffee available

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on the domestic market, determining the amount of caffeine intake is of great importance (Kaloper, 2017).

Caffeine is the most popular natural stimulant, a plant alkaloid that has a positive effect on mental and physical functions. Although it is found in about 60 plant species, caffeine is most often ingested by consuming coffee, tea, Coca-Cola, products with guarana extract, and more recently by consuming energy drinks, the consumption of which is continuously growing. However, due to the potential health risks of caffeine consumption, it is extremely important to take care of the amounts consumed (Ivančić, 2017).

Caffeine, a natural alkaloid in coffee

Caffeine is an alkaloid that we consume daily in the form of coffee, tea, cola drinks and chocolate. Caffeine has been found (identified) in about 60 plant species, and is most prevalent in coffee, tea, and cocoa beans. Caffeine is the most widely used psychoactive substance in the world (Ogawa et al., 2007). It belongs to a group of compounds known as alkaloids. It is one of the most diverse groups of secondary metabolites found in living organisms and has a wide range of types of structures, biosynthetic pathways, and pharmacological activities (Roberts, 2013). Alkaloids are complex organic heterocyclic and basic compounds that contain nitrogen in their structure and after introduction into the body exhibit specific pharmacological and toxicological action.

Caffeine is the most widely used central nervous system (CNS) stimulant in the world (Institute of Medicine Staff, 2001).

Although 400 mg of caffeine a day is a "safe" amount for adults, it does not have to be "safe" for children and adolescents (Health Canada, US. Food and Drug Administration (FDA 2012), European Food Safety Authority, U.S. Dietary 4 Guidelines for Americans). The biggest problem with caffeine is that it will dehydrate the body anyway, and that dehydration can be serious, even deadly. In addition, caffeine is addictive, so the more it is consumed, the more it is needed to maintain the "excited" state of the body (Parker, 2008).

The mass of caffeine in one cup of coffee (volume about 240 mL) is usually about 80 mg, although it can vary from 5 to 190 mg depending on the method of preparation as well as depending on the size of the cup itself. Regular coffee consumers usually take in about 256 mg of caffeine a day (on average 4.3 mg of caffeine per 1 kg of body weight (bw or BW) for a person who weighs 60 kg), although this number for 90 % of coffee consumers is between 5 and 7 mg per kg body weight. Most studies indicate that caffeine intake of 400 mg or less per day does not have a negative impact on health for most consumers. Since caffeine occurs naturally in tea and coffee, it is difficult to determine the maximum allowable levels of caffeine for them. But for products where caffeine is not naturally present, there are some limitations in most countries. Interestingly, there is no upper limit for caffeine in the European Union for products with the same, but products containing more than 150 mg/L must be mentioned to have a high caffeine content (Kaloper, 2017).

Different methods of preparing a coffee beverage affect the caffeine content in the beverage. For example, filter coffee contains 0.67 g of caffeine in 1 liter of beverage, flour 2.36 g/L, and a beverage obtained by brewing ground coffee, such as "Turkish" coffee, contains 0.57 g/L of caffeine. When preparing an espresso beverage, the extraction of caffeine from coarsely ground coffee in the filtration process is not complete. The reason for this is the short period of time for the separation of caffeine from the cell structure. Therefore, the concentration of caffeine in espresso varies from 1.2 g/L to 4 g/L, depending on the size of the cup and the composition of the mixture (Clarke and Vitzthum, 2001).
The acute toxicity of caffeine is not well defined, but it considered to be worrying more than 10 grams of caffeine per day for adults, while in most countries does not recommend consuming more than 450 mg of caffeine a day (Heckman et al., 2010).
Health Canada has recommended a maximum caffeine intake of 2,5 mg/kg body weight / day for children under 12 (Health Canada 2012).
Bühler et al. in 2013, they conducted a study on the content of caffeine in food and beverage samples. For the first time, a tool (a questionnaire with a calculation program) was developed and validated to assess caffeine intake among adolescents and young adults. This has been shown to be applicable in surveys of more than 200 students. The average caffeine intake during the working day was between 105 mg and 130 mg. Coffee was the main source.
Total daily caffeine intake has remained stable in the last 10–15 years, and coffee, tea and soft drinks are the most important caffeine sources (Verster, Joris C et al., 2018).
Although there are cases where consumption of very high dosages of caffeine has led to seizures, transient cardiovascular problems, and even deaths (Cappelletti et al. 2018., vKRR et al. 2018.) comprehensive reviews have concluded that consumption of < 400 mg/day is generally safe, enhances certain aspects of mental, physical, and occupational performance, and may confer other health benefits (Dietary Guidelines Advisory Committee, 2016., Nawrot et al., 2003., Wikoff et al. 2017).

Scientific Opinion on the Safety of Caffeine (EFSA, 2015)

At the request of the European Commission, EFSA's Scientific CommiBWee on Dietary Products, Nutrition and Allergies (NDA) has developed a Scientific Opinion on the Safety of Caffeine (EFSA, 2015), which assessed acute and daily caffeine intake that does not pose a risk to general healthy population.
The main source of caffeine in the diet of adults is coffee, it is estimated that the daily intake in EU member states ranges from 0.5 to 4.6 mg of caffeine / kg bw, in adolescents it is chocolate (0.4 to 1.4 mg / kg bw), and in children chocolate, teas and soft drinks (from 0.2 to 2 mg / kg bw).
Given the available data from studies on the effects of caffeine on the cardiovascular system, central nervous system (e.g., insomnia and nervousness) and possible risks to fetal health in pregnant women, the EFSA CommiBWee reached the following conclusions:

Table 1. *Different doses of caffeine intake and health risks*

A single dose of caffeine of 100 mg (about 1.4 mg / kg bw)	may affect the length of sleep in some adults, especially when consumed just before bedtime
Daily intake of up to 400 mg (about 5.7 mg / kg bw)	does not pose a risk to the health of healthy adults, except pregnant women
Daily intake of caffeine from all sources up to 200 mg	does not pose a risk to the fetus, i.e., pregnant, and lactating women
A single dose of caffeine of 200 mg (about 3 mg / kg bw) from all sources	does not pose a risk to the health of healthy adults

Consumption of other ingredients of energy drinks in concentrations common to such beverages does not affect the safety of consuming a single dose of caffeine up to 200 mg.

Materials and methods

Laboratory analyses

The analysis of caffeine content was performed in the laboratory of the Faculty of Teacher Education of the University "Džemal Bijedić" in Mostar by the method of the High-performance liquid chromatography (HPLC). The analysis was performed on a chromatograph model SHIMADZU USA: SCL - 10A VP during 2019 on 10 samples of coffee beverages. For this purpose, 10 different samples of coffee and coffee beverages were selected from the market.

Samples

As material, 10 samples of coffee from the stores of the city of Mostar were used:

1. Sample No.1: JACOBS 3 IN 1, Country of origin: Amsterdam, The Netherlands, Usable until: 14.11.2020, Amount: 15.2 g
2. Sample No.2: NESCAFE MACCHIATO 3 IN 1, Country of origin: Romania, Usable until: 2 months 2020., Quantity: 15.00 g
3. Sample No.3: NESCAFE 3 in 1 Classic, Country of origin: Romania, Usable up to: 6 months. 2020, Amount: 17.5 g
4. Sample No.4: NESCAFE classic strong and rich, Country of origin: Spain, Usable until: 4 months 2020., Quantity: 2 g} × 2
5. Sample No.5: NESCAFE 3 In 1 strong, Country of origin: Hungary, Usable until: 10 months 2020., Quantity: 18 g
6. Sample No.6: Franck I LOVE CAFE 3 IN 1 INSTANT MIX, Country of origin: Croatia, Usable until: 24.10.2020, Quantity: 18 g
7. Sample No.7: El Cafe classic 3 in 1, Country of origin: Turkey, Usable until: 12 months 2020., Quantity: 18 g

8. Sample No. 8: Franck CREMA, Country of origin: Croatia, Usable until: 15.12.2019., Quantity: 9 g

9. Sample No. 9: Zlatna Džezva, Country of origin: BiH, Usable until: 4 month 2020., Quantity: 8 g

10. Sample No.10: Grand black and easy, Country of origin: Serbia, Usable until: 11.04.2020., Quantity: 8 g.

Procedure

Preparation of the mobile phase

We prepared a 20:80 (v / v) solution of methanol, and chromatographic conditions were set. LC pump flow was set as follows: 1 mL / min., UV wavelength VIS 278nm, injection volume 10 ql 4, column C 18250x4.6 mmi.d.

After setting all the conditions, the pump was started, the method was loaded and the mobile phase was left to pass through the column for at least 10 min. The baseline had to be stable before injecting standards and samples.

1. *Preparation of standard solutions*

A baseline caffeine standard of 0.02 g was prepared by dissolving the analytical standard of caffeine in 100 mL of deionized water.

Solutions of standard concentrations were used for calibration purposes and were prepared with contractions of 5, 20, 15, 20 and 25 ppm by diluting the stock solution in a 100 mL volumetric vessel.

2. *Sample preparation*

For the purpose of the analytical balance the weight of 3-5 g of the sample has to be weighted.

This amount of weighted sample has to be transferred to a 250 mL beaker and poured with 200 mL of boiling water and allowed to stand in a covered vessel for 5 min. Then it should be filtered into a 250 mL beaker.

3. *Determination of caffeine content in samples*

A series of standards and samples in triplicates in the HPLC system has to be injected and recorded in the chromatograms.

The height and area of the peaks from the chromatograms was determined. Based on the data for standard solutions, two calibration curves were constructed: a) dependence of the peak area (cm 2) on the concentration (mg / mL) and b) dependence of the peak height (cm) on the concentration (mg / mL). The direction equation for both calibration curves and the caffeine content in the samples were determined (Figure 1).

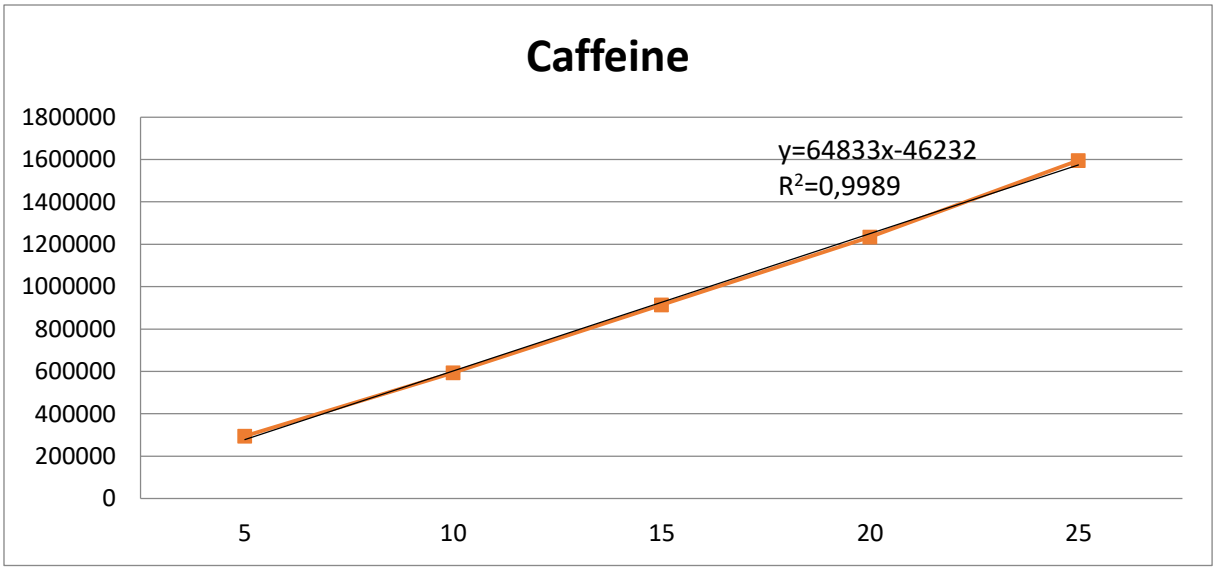

Figure1. Calibration curve of caffeine standard readings

Survey on coffee consumption

The survey was conducted in B&H on a sample of 328 respondents aged between 16 to more than 60 years old. Internet surveys were used in the form of filling out a questionnaire consisting of 9 questions. The survey on the frequency of caffeine consumption and knowledge of the negative effects of caffeine was conducted in 2019.

Table 2. *Questionnaire*

1. Gender	M Ž
2. Age	a)10-15 b) 16-20 c) 21-30 d) 31-40 e) 41-50 f) 51-60 g) more than 60
3. Degree of education	a) primary school b) high school c) bachelor e) Master of Science f) Doctor of Science
4. Status	a) student b) unemployed c) employed d) retiree
5. How often do you consume coffee or instant coffee drinks?	a) every day b) 3-5 times a week c) 1-3 times a week e) 3-5 times a week

6. The amount of coffee you drink per day (suppose one serving is 100 mL)	a. 100 mL b. 150 mL c.200 mL d. 300 mL e.400 mL
7. What is your reason for consuming coffee or instant coffee drinks?	a. improving physical endurance b. maintaining alertness c. increase mental and cognitive abilities d. I like the taste e. out of habit
8. Are you aware of the harmful effects of high caffeine intake?	a) Yes b) No.
9. What amount of daily caffeine intake do you consider harmful?	a) 50 mg b) 100 mg c) 200 mg d) 300 mg e) 500 mg f) 1000 mg d) I don't know

The frequency of consuming coffee and coffee beverages in the wider area of Mostar was determined through the realization of the set goals. The results of the survey indicated how familiar the respondents are with the amounts of caffeine that is harmful to acute and chronic coffee intake.

EDI and EWI of caffeine consumption

The formula recommended by the US EPA (1992) was used in the calculation of the average daily and weekly intake. The calculation of the risk assessment due to the exposure of the examined population was done based on a survey that included 328 respondents.

EDI = CxUd / BW

EWI = CxUW / BW

C- average concentration of caffeine

The calculation of EDI and EWI was done based on the responses from the survey on average daily acute (228,01mL) and average weekly chronic (852,88mL) intake.

Ud – 228,01 mL *

Uw – 852,88 mL *

BW- body weight for adults according to WHO

* Intake of coffee drinks daily and weekly based on survey responses.

Results and discussion

The caffeine content in the samples ranged from 205.96 – 701.68 g /L. The highest content of caffeine was read in the sample of coffee No. 9 Zlatna džezva 701.68 g/L, and in the sample no. 8 Franck creme 656.22 g/L. This is followed by sample no. 4 Nescafe strong and rich 512.84 g/L, sample no. 10 Grand black and easy 481.87 g/L, sample no. 1 Jacobs 490,15 g/L, sample no. 7 El Caffe classic 383.90 g/L, sample no. 5 Nescafe strong 371.63 g/L, sample no. 3 Nescafe 3in1 classic 282.77 g/L, sample no. 6 Franck 3in1 247.22 g/L, and sample no. 2 Nescafe 3in1 205.96 g/L.

In a study of the caffeine content of espresso coffee, Desbrow et al. in 2012, 131 samples of espresso coffee were collected in Australia, where caffeine values ranged from 107±37 mg per serving, and caffeine concentrations were 2550±1030 mg/L.

Table 3. *Caffeine reading on HPLC (g /L), EDI and EWI*

Samples	**x(g/L)**	**g/mL**	**EDI mg / kg body weight**	**EWI mg / kg body weight**
1.Jacobs	490.15	0.49	1.82	29.02
2.Nescaffe 3u1	205.96	0.206	0.76	12.20
3.Nescaffe 3u1 classic	282.76	0.283	1.05	16.76
4.Nescaffe strong and rich	512.84	0.512	1.90	30.33
5.Nescafee strong	371.63	0.372	1.38	2.04
6.Franck 3u1	247.22	0.247	0.92	14.63
7.El Caffe classic	383.90	0.384	1.43	22.75
8.Franck creme	656.22	0.656	2.44	38.86
9.Zlatna džezva	701.68	0.702	2.61	41.58
10.Grand black and easy	481.87	0.482	1.79	28.55

The average daily intake of EDI was done based on a survey from all coffee beverage samples that do not exceed the recommended values prescribed by EFSA (2015) about 3 mg/kg bw. EDI values ranged from highest to lowest Zlatna džezva (2.60 mg/kg BW) > Franck creme (2.44 mg/kg BW) > Nescafe strong and rich (1.90 mg / kg BW) > Jacobs (1.82 mg/kg BW) > Grand black and easy (1.79 mg/kg BW) > El Caffe classic (1.43 mg/kg BW) > Nescafe strong (1.38 mg/kg BW) > Nescafe 3in1 classic (1.05 mg /kg BW) > Franck 3in1 (0.92 mg/kg BW) > Nescafe 3in1 (0.76 mg/kg BW).

EWI values followed the same principle. Zlatna džezva (41.58 mg /kg BW) > Franck creme (38.86 mg kg BW) > Nescafe strong and rich (30.32 mg / kg BW) > Jacobs (29.02 mg / kg BW) > Grand black

and easy (28.55 mg/kg BW) > El Caffe classic (22.75 mg /kg BW) > Nescafe strong (22.03 mg/kg BW) > Nescafe 3in1 classic (16.76 mg/kg BW) > Franck 3in1 (14.63 mg/kg BW) > Nescafe 3in1 (12.20 mg / kg BW).

In 2015, Shatha et al investigated the caffeine content of beverages commonly consumed in Jordan. 167 samples were collected from the market in Amman. The caffeine content was determined by high performance liquid chromatography (HPLC). Caffeine concentrations ranged from 12.37 to 194.61 mg/100 mL in coffee samples which is in correspondence with the study conducted in this research. Espresso coffee and Turkish coffee had the highest caffeine content (194,6 and 146,6 mg/100 mL). This is the first study on caffeine content in the Arab world.

The intake of caffeine in the diet of the Hungarian population was estimated based on data from the National Nutrition Survey in 2009. The daily intake of caffeine in adult Hungarian men and women was 147 ± 6,2 mg per capita and 138 ± 4,2 mg per capita. There was no significant gender difference. The eldest men and women consumed significantly less caffeine than people aged between 35 to 64 years old. The main sources of caffeine are coffee and tea with 58-59 % and 35-37 % of the total intake in men and women (Lugasi et al. 2015).

Based on statistical data processing by one-factor analysis of the significance of differences (ANOVA), a statistically highly significant difference in caffeine content was found in different samples of coffee beverages (F>F crit.) F = 8.30; p = 4.57E-05. After that, the Tukey - Kramer test was performed to confirm the statistical significance in the measurements. Sample no.9. compared to all other samples showed a statistically highly significant difference in caffeine content

Table 4. *ANOVA statistical significance level of 0,05*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	74.72148	9	8.302387	8.302387	4.58E-05	2.392814108
Within Groups	20	20	1			
Total	94.72148	29				

The survey was conducted on a total of 328 respondents, 62 men (19 %) and 266 women (81 %). As many as 72 % (238) of the respondents are aged 21-30, i.e., younger.

Out of a total of 328 respondents, 179 or 54 % have a bachelor's degree, while 16 % have a master's degree in survey research, and it can be assumed that their knowledge of caffeine and its harmful effects on health should be at an enviable level.

As many as 74 % of respondents consume coffee every day, which shows that coffee is a beverage that is often consumed in our country, 241 respondents every day (74 %)

Considering that the trend of drinking coffee is very popular in Bosnia and Herzegovina, and that coffee is drunk from traditional cups, cups of various servings (from 50 mL - 200 mL), this question is formulated under the assumption of one serving of 100 mL. Most respondents drink 200 mL of coffee a day.

In order to get information of the reasons to why they drink coffee or instant coffee drinks, the most answers were related to habits, as many as 119 of them. 99 respondents said the reason for consumption was because they like the taste, and 57 to maintain alertness.

In the research of the ISIC Institute for Scientific Information on the habit of consuming coffee at the workplace from 2017, they state that the main reasons for drinking coffee at work were: taste (56 %); use time for breaks and rest to drink or prepare coffee (40 %); and to keep them awake (29 %). Given that more than a quarter of respondents said they drink coffee to feel more alert, this indicates that people choose coffee to keep them awake at work.

Regarding the question of how much the respondents are aware of the harmful effects of high caffeine intake, most of them are familiar with the harmful effects, which is 214 respondents or 65 %. Considering that the largest number of respondents were aged between 21-30, and that they have a university degree, it is a devastating fact that 35 % are not aware of the negative effects of caffeine on human health.

118 respondents answered that they do not know what daily amount of caffeine is harmful to health, which is 36 %. It is also a devastating fact that a large number of respondents are younger and highly educated people who consume the drink daily without knowing the harmful consequences of high caffeine intake. This is certainly a worrying fact because in 2015, EFSA issued a statement that a single dose of 200 mg of caffeine from all sources does not pose a risk to the health of healthy adults. (EFSA, 2015).

In Brazil, daily caffeine intake per person is estimated at 115.7 mg, ranging from 84.7 mg for children and adolescents aged 10 to 13, to 139.8 mg for the uneducated. The percentage of people whose daily caffeine intake is higher than 400 mg is up to 3.0 %, according to age groups. Men and individuals living in the northeastern or southern part of the region or in the states of Minas Gerais, Rio de Janeiro and Espírito Santo are likely to consume higher amounts of caffeine. The main food sources are coffee (63.1 %) and coffee with milk (24.9 %), soft drinks Cola (3.6 %) and yerba mate (1.9 %) Alan G. et al. 2016.).

Conclusion

A study on the frequency of consumption of coffee and caffeinated beverages was conducted during 2019 using the method of empirical research, descriptive research and HPLC method (high performance liquid chromatography method) to determine the caffeine content in various coffee samples.

The caffeine content in the samples ranged from 205,96 – 701,68 g /L. The highest content of caffeine was read in the sample of coffee Zlatna džezva 701,6755 g/l, and in the sample Franck creme 656,22 g /L.

Based on statistical data processing by one-factor analysis of the significance of differences (ANOVA), a statistically highly significant difference in caffeine content was found in different samples of coffee beverages (F > F crit.) F = 8,30; p = 4,57E-05.

EDI values ranged from highest to lowest Zlatna džezva (2,60 mg / kg BW) > Franck creme (2,44 mg / kg BW) > Nescafe strong and rich (1,90 mg / kg BW) > Jacobs (1,82 mg/kg BW) > Grand black and easy (1,79 mg/kg BW) > El Caffe classic (1,43 mg / kg BW) > Nescafe strong (1,38 mg / kg BW) > Nescafe 3in1 classic (1,05 mg / kg BW) > Franck 3in1 (0,92 mg / kg BW) > Nescafe 3in1 (0,76 mg / kg BW).

The average daily intake of EDI was done by the basic research survey of all samples of coffee beverages that do not exceed the recommended values determined by the EFSA (2015), about 3 mg / kg BW.

A total dietary study has not been conducted in Bosnia and Herzegovina, and these data suggest the need for the same. There are many different types of coffee drinks, as well as the ways of their preparation and ritual of consumption, which primarily depends on the tradition and culture of society, but also on the habits of consumers. This data can serve as a basis for some other research in the field of food safety.

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