Quick estimation of dietary exposure to heterocyclic aromatic amines and acrylamide in a Croatian female population

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Summary

This study used a quick interview and calculation for a preliminary estimation of dietary exposure to heterocyclic aromatic amines (HAA) MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), as well as acrylamide (AA) in 94 adult women from eastern Croatia. Subjects were administered a questionnaire to obtain information on their anthropometric and socio-economic characteristics, and on their dietary habits. Intake of main sources of HAA and AA was determined and combined with literature data on their food levels. Both high and low estimates were calculated by the use of maximum and minimum published levels. The low estimate approach produced mean daily exposures to MeIQx (0.93 ± 0.77 ng·kg^{-1} body weight, bw) and PhIP (2.34 ± 2.49 ng·kg^{-1} bw), as well as a provisional total HAA intake of 4.43 ng·kg^{-1} bw per day, that fit better within the range of results reported by other authors. Similarly, the low estimate of AA daily exposure (122.66 ± 60.00 ng·kg^{-1} bw) was below the lower end of the range determined by the European Food Safety Authority but more plausible considering other published levels.

Keywords

acrylamide; heterocyclic aromatic amines; dietary intake; women; exposure

Studies have indicated neurotoxicity and carcinogenicity as major potential consequences of exposure to acrylamide (AA) [1]. These adverse affects are typically seen at higher doses with which experimental animals were treated, although neurotoxicity has also been observed in the context of occupational and accidental exposures [2]. In addition to a wide range of industrial and laboratory applications of AA, it can be formed in food in a Maillard-type reaction between amino acids and reducing saccharides [3]. According to an European Food Safety Authority (EFSA) report [4], maximum levels were determined in potato crisps and substitute coffee, and major contributors to dietary intake in adults were fried potatoes, coffee and soft bread. Since it is a genotoxic carcinogen [5], a number of epidemiological studies investigated the relationship between AA dietary exposure and cancer. Several prospective studies determined an increased risk of cancer at different sites, e.g. endometrial cancer [6], ovarian cancer [6], renal cell carcinoma [7], breast cancer [8]. EFSA Scientific Colloquium on acrylamide carcinogenicity noted incosistent findings and concluded that more prospective studies are needed [9]. Nevertheless, recent reviews of epidemiologic studies concluded that no link between dietary AA exposure and cancer existed [10, 11].

Another group of problematic products of Maillard reactions between free amino acids, creatin(in)e and hexoses are heterocyclic aromatic amines (HAA), which can primarily be detected in cooked meat [12]. A number of factors may influence their formation in foods, including cooking time and temperature, cooking methods as well as the type of food that contains precursors and inhibitors [13, 14]. Generally, the levels are higher in cooked meats than in fish. The most abundant and possibly carcinogenic HAA are 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyrid-

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ine (PhIP) [15]. Several large epidemiologic studies determined a positive correlation between red meat intake and cancer risk [16 and references therein]. This may be partly explained by the presence of mutagenic HAA in meat.

Except for one tentative calculation of AA exposure [17], there are no published data on either AA or HAA dietary intake in Croatia. Therefore, the aim of this work was to make a pilot estimation of dietary exposure to AA and selected HAA in adult women from eastern Croatia. Specifically focusing on a segment of female population most cognizant of local cooking practices and habits, the study aimed to provide information relevant for this unexplored public health issue. Exposures similar to other reports, in particular European ones, are hypothesized, which would justify the use of this inexpensive, quick method in preliminary risk assessment.

SUBJECTS AND METHODS

Subjects

Ninety-four healthy middle-aged and elderly women were asked to take part in the study and their informed consent was obtained. Participation rate was 94%. The subjects were recruited from randomly selected households, which were uniformly spread across several chosen towns and villages in Baranja (eastern Croatia): Beli Manastir, Suza, Kneževi Vinogradi and Lug. Nineteen percent of subjects were from an urban setting. In addition to a questionnaire on cooking practices and habits, subjects also provided basic socio-economic information and were subjected to anthropometric measurements to determine the body mass index (BMI). The mean age was 55.5 ± 13.2 years (range 30–85 years); the mean BMI was 26.7 ± 4.7 kg·m^{-2} (range 17.2–40.3 kg·m^{-2}). The proportion of normal weight subjects was 38.3%, 36.2% were overweight, 23.4% were obese, and 2.1% subjects were undernourished. Questionnaire data were used to divide subjects according to economic and education status. The following monthly income per household member categories were used: ≤ 259 € (33%), 260–399 € (26%), ≥ 400 € (41%). Education levels were: unfinished primary school (14%), primary school (41%), high school (31%), and university (14%).

Questionnaire

The purpose of the questionnaire was to assess types of food, frequency of consumption over a 7-day period, and cooking techniques characteristic for the chosen population. An example of the form used by interviewers is presented in Tab. 1. HAA intake was estimated on the basis of intake of cooked meat: poultry, pork, beef and fish. AA intake was calculated from the consumption of its major sources according to the EFSA report [4]: potato crisps, French fries and home-cooked potato products (deep-fried or oven-baked), biscuits (cookies, crackers, wafers), bread and bakery products, breakfast cereals (including

Tab. 1. Questionnaire used to estimate nutritional habits and cooking methods.

Please describe your usual consumption of food items listed below over a 7-day period:				
Meat	daily	2-3 x per week	once a week	never
Potatoes	daily	2-3 x per week	once a week	never
Potato crisps	daily	2-3 x per week	once a week	never
Cookies	daily	2-3 x per week	once a week	never
Bread	daily	2-3 x per week	once a week	never
Bakery products	daily	2-3 x per week	once a week	never
Corn flakes	daily	2-3 x per week	once a week	never
Breakfast cereals	daily	2-3 x per week	once a week	never
Coffee	daily	2-3 x per week	once a week	never
How many cups of coffee do you drink per day?				
Which technique do you most often use to cook meat?	Pan-frying	Grilling	Oven-broiling	Stewing / Boiling
Which type(s) of meat do you most frequently consume?	Pork	Beef	Poultry	Fish
Which technique do you most often use to cook potatoes?	Roasting / Baking	Frying	Boiling / Steaming	

Tab. 2. Medium portion sizes for different meats and the levels of selected heterocyclic aromatic amines dependent on cooking technique.

Meat type	Medium portion [g]	Grilling				Oven-broiling				Pan-frying			
		MeIQx [μg·kg^{-1}]		PhIP [μg·kg^{-1}]		MeIQx [μg·kg^{-1}]		PhIP [μg·kg^{-1}]		MeIQx [μg·kg^{-1}]		PhIP [μg·kg^{-1}]	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Poultry	70	9	9	27	480	3	3	6	150	0.4	3	0.5	70
Pork	80	0.2	0.4	2.5	30	1.7	1.7	2.1	7.1	2.6	5.4	2.3	13.4
Beef	50	4.6	4.6	16.8	16.8	1.6	1.7	2.1	7.1	0.34	7.3	0.01	32
Fish	150	0.03	0.03	0.5	7.4	4.6	4.6	1.7	23	0.01	6.44	0.01	18

High – the highest reported MeIQx and PhIP levels for a specifically cooked meat; Low – the lowest detected levels. Literature sources for heterocyclic aromatic amines levels: [13, 18]. Medium portion sizes for different meat types were taken from food frequency questionnaire developed by Faculty of Food Technology, Osijek, Croatia.

corn flakes) and coffee drink. The subjects were encouraged to detail approximate proportions of consumed type(s) of meat and potato dishes as well as the used cooking techniques.

Calculation of HAA intake

The mean dietary intakes of MeIQx and PhIP were estimated on the basis of average weekly intake of a meat type, and MeIQx and PhIP levels in different kinds of cooked meat.

The former was calculated by multiplying the medium portion size by the consumption frequency reported by the subjects. Medium portion sizes for different meats (Tab. 2), originally from a food frequency questionnaire developed by our group at Faculty of Food Technology, Osijek, Croatia, were determined on the basis of several measurements of a food item prepared in separate households. Finally, estimation of MeIQx and PhIP intake with consumed meat included approximation of quantities of each meat type prepared by specific cooking techniques. Subjects reported the most frequently used cooking technique and it was assumed that 2/3 (i.e. 67%) of all meat was cooked in that fashion. Since MeIQx and PhIP levels in cooked meats vary largely even if prepared by the same technique, a two-pronged approach was taken. Separate calculations were made using values at the high end as well as those at the very low end of the measured content range from two review articles [13, 18]. The used levels are presented in Tab. 2. Considering that boiling and steaming did not lead to HAA formation in any of the major meat types, if the most used technique was either pan-frying, oven-broiling or grilling, the rest of the meat quantity was assumed to have been boiled or steamed and to have contained no HAA. In contrast, if most consumed meat type was boiled/steamed, the remaining 33% was supposed to have been cooked by a technique having a diametrically opposite effect on HAA formation. In other words, a technique producing greatest HAA levels was used in calculations. For example, if a subject consumed fish 2–3 times a week and reported that the greatest quantity of meat was pan-fried (67%), the calculation of high estimate daily MeIQx intake proceeded as follows: 2.5 (frequency of consumption per week) × 150 g (medium portion) × 0.67 (fraction of meat prepared by frying) × 6.44 μg·kg^{-1} (MeIQx content in pan-frying fish) = 1618 ng per week = 231 ng per day.

General equation form (Eq. 1) was repeated for each consumed meat type and their sum represented total daily intake of MeIQx and PhIP, respectively.

$$DI_{\mathrm{MeIQx/PhIP}} = \frac{W_{\mathrm{m}} \cdot M_{\mathrm{m}} \cdot F_{\mathrm{m}} \cdot M_{\mathrm{HAA}}}{7} \quad (1)$$

where $DI_{\mathrm{MeIQx/PhIP}}$ is daily intake of MeIQx and PhIP (in nanograms), W_{m} is consumption frequency of a meat type in a week, M_{m} is medium portion size (in grams) of a meat type, F_{m} is fraction of meat prepared by a specific cooking technique, M_{HAA} is mean content of MeIQx or PhIP (in nanograms per gram) in meat.

Calculation of acrylamide intake

Dietary intake of AA was calculated from average weekly intake of foods listed in Tab. 3, and their mean AA levels according to the literature.

As described for HAA intake above, weekly food intake was determined from medium portion size for the food items multiplied by the reported consumption frequency. The used medium portion sizes given in Tab. 3 were obtained from the same source as described for HAA. Since comprehensive data on AA content in Croatian foods are lacking, the mean AA food levels used in cal-

Tab. 3. Medium portion sizes for foods that are main contributors to acrylamide intake and its mean content.

Foods	Medium portion size [g]	Acrylamide level [$\mu g \cdot kg^{-1}$]	
		Low	High
Potato, fried or baked	170	69.2	919.3
Potato crisps	40	195.7	1941.7
Cookies	50	67.4	800
Bread	100	10.7	272.8
Breakfast cereals	80	89.5	454.3
Coffee drink	100	154.1	1023.4

Low and high reported mean acrylamide levels are 25th and 95th percentile levels, respectively, as reported by EFSA [4]. Medium portion sizes for different foods were taken from a food frequency questionnaire developed by Faculty of Food Technology, Osijek, Croatia.
* – acrylamide content in dry coffee powder; 100 g drink contains approximately 7 g powder.

culations were from the report by EFSA [4]. For potato, cooking technique was important, so if the most frequently used techniques were roasting, baking or frying, it was assumed that 2/3 of all potato dishes were prepared by these techniques, and the calculated quantity was multiplied by AA level in fried potatoes (Tab. 3). The rest was assumed to have been prepared by boiling, which produces no AA. Converse assumption and calculations were made if subjects reported that potato was most frequently boiled. Daily intake of AA from potato dishes (Eq. 2) was then added to the sum of AA contributed by other major food sources (Eq. 3) to obtain total daily AA intake.

$$DI_{\mathrm{AA-CC}} = \frac{W_{\mathrm{i}} \cdot M_{\mathrm{i}} \cdot M_{\mathrm{AA}}}{7} \quad (2)$$

where $DI_{\mathrm{AA\text{-}CC}}$ is daily intake of AA from cereal products and coffee (in nanograms), W_{i} is consumption frequency of a food item in a week, M_i is medium portion size (in grams), M_{AA} is mean content of AA (in nanograms per gram) in food item.

$$DI_{\mathrm{AA-P}} = \frac{W_{\mathrm{p}} \cdot M_{\mathrm{p}} \cdot F_{\mathrm{p}} \cdot M_{\mathrm{AA}}}{7} \quad (3)$$

where $DI_{\mathrm{AA\text{-}P}}$ is daily intake of AA from potato dishes (in nanograms), W_{p} is consumption frequency of a potato dish in a week, M_{p} is medium portion size (in grams), F_{p} is fraction of potato prepared by a specific cooking technique, M_{AA} is mean content of AA (in nanograms per gram) in potato dish.

Statistical analyses

Calculation of descriptive parameters, determination of Pearson's correlation coefficient and least significant difference (LSD) test for post-hoc comparisons were performed by Statistica 7.0 (StatSoft, Tulsa, Oklahoma, USA) and Microsoft Office Excel 2010 (Microsoft, Redmond, Washington, USA). Differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Results for HAA and AA daily dietary intake are presented in Tab. 4.

The mean daily MeIQx intake differed by approximately 30% between the best and worst case scenarios. The high estimate was similar to the mean reported for US population in a study by Nowell et al. [19], i.e. 0.09 $\mu g \cdot d^{-1}$ (exposures reported by other authors in nanograms per kilogram of body weight (bw) are given in Tab. 5). Notably, the mentioned research group utilized a more elaborate questionnaire than the one used in this work. It included food models to estimate portion size and photographs of foods cooked to different degrees of doneness to estimate food preparation techniques. Layton et al. [26] estimated a daily exposure to MeIQx, which is two times higher than the present high estimate (Tab. 5), while Keating and Bogen [27] determined a very similar average exposure in a US white female population.

The high estimate PhIP intake was 35-times higher than the analogously calculated MeIQx intake (Tab. 4). The large standard deviation of the mean stems from extensive variations in the subjects' intake of different meat types. For example, the highest PhIP intakes were invariably associated with poultry, which has been reported to be the most frequently consumed meat type. The effect was compounded by comparatively high PhIP contents in poultry, regardless of the cooking technique (Tab. 2).

Methodology used in this work did not account for preparation temperature, cooking time or the degree of doneness and these factors can result in large differences in PhIP content. According to Keating and Bogen [27], PhIP was much more sensitive to changes in cooking temperature and time compared to MeIQx. This can also explain the vast difference between the low and high estimate of PhIP intake (Tab. 4). Additionally, PhIP content in foods is up to several 100-fold higher than the levels of MeIQx and other HAA in common meats, and this will always lead to higher in-

Tab. 4. Daily dietary intake, age-dependent distribution and exposure to selected heterocyclic aromatic amines and acrylamide in a female cohort from eastern Croatia.

Daily intake [μg·d^{-1}]						
	Heterocyclic aromatic amines				Acrylamide	
	MeIQx		PhIP			
	High estimate	Low estimate	High estimate	Low estimate	High estimate	Low estimate
Mean ± SD	0.08 ± 0.06	0.06 ± 0.05	2.88 ± 2.74	0.16 ± 0.16	119.60 ± 41.91	8.41 ± 4.13
Median	0.07	0.05	2.25	0.10	119.19	7.71
Range	0.01–0.32	0.003–0.32	0.05–16.80	0.003–0.95	47.74–238.16	1.87–31.11
Age group means						
30–49 years (n = 48)	0.09 ± 0.06	0.06 ± 0.06	2.81 ± 3.38	0.15 ± 0.19	111.07 ± 43.28	8.42 ± 3.40
50–69 years (n = 31)	0.07 ± 0.04	0.06 ± 0.04	2.75 ± 2.09	0.15 ± 0.12	125.32 ± 43.29	8.94 ± 4.86
70–89 years (n = 15)	0.09 ± 0.08	0.07 ± 0.07	3.46 ± 3.16	0.21 ± 0.23	118.90 ± 32.97	6.69 ± 2.30
Daily intake per body weight [ng·kg^{-1}]						
Mean ± SD	1.21 ± 0.85	0.93 ± 0.77	42.41 ± 40.71	2.34 ± 2.50	1 749.14 ± 651.76	122.66 ± 60.00
Median	1.01	0.83	29.72	1.66	1 813.99	116.62
Range	0.19 – 5.03	0.03 – 4.30	0.81 – 229.51	0.04 – 13.51	652.19 – 4 057.20	27.62 – 397.80

Number of participants n = 94. SD – standard deviation.

Tab. 5. Exposure to selected heterocyclic aromatic amines and acrylamide in different countries.

Country		n	Sex	Mean daily HAA exposure [ng·kg^{-1}]			Reference
				MeIQx	PhIP	Total HAA	
Croatia	Low estimate	94	F	0.93	2.34	4.43	This study
	High estimate			1.21	42.41	60.52	
Malaysia[a]		600	50% M	n. a.		8.84	[21]
Singapore[b]		497	51% M	0.21	0.49	0.83	[23]
Sweden		544	51% M	1.03	1.03	2.29	[24]
USA[c]		380	66% M	1.12	1.92	3.11	[19]
		3 563	n. a.	2.61	16.64	25.51	[26]
		4 955	F	1.4	9.2	13.5	[27]

Country		n	Sex	Mean daily acrylamide exposure [ng·kg^{-1}]	Reference
Croatia	Low estimate	94	F	122.66	This study
	High estimate			1 749.14	
Australia		13 858	n. a.	450	[28]
China		n. a.		380	[29]
European Union		36 458	n. a.	310–1 070	[4]
France		1 918	n. a.	430	[30]
Poland		2 893[d]	F	320	[31]
Sweden		1 200	n. a.	500	[32]
The Netherlands		6 250[e]	n. a.	480	[33]

HAA – heterocyclic aromatic amines, F – female, M – male, n. a. – data not available
a – mean intake in nanograms was divided by the mean weight of Malaysian adults (18 years and over) reported in [20], b – mean intake in nanograms was divided by the mean weight of Singaporean Chinese adults (40–79 years) reported in [22], c – mean intake in nanograms was divided by the mean weight of US adults (20 years and over) reported in [25] and adjusted for percentages of male and female control subjects in [19]: 88.3 kg × 0.66 + 74.7 kg × 0.34, d – total number of adults; e – age group 1–97 years.

takes. However, the disparity was much weaker for the low estimates of HAA intake in the present cohort (Tab. 4), which can again be explained by the inclusion or absence of poultry (i.e. chicken meat) in the diet. The low PhIP estimate was identical to 0.16 $\mu g \cdot d^{-1}$ of NOWELL et al. [19]. Among other reports listed in Tab. 5, LAYTON et al. [26] determined the highest exposure, and while it may well be one of the highest reported PhIP intakes in the literature, it is still two times lower than the high estimate in the present study (Tab. 5).

Obviously, different populations were investigated in the abovementioned reports and their nutritional habits might have differed considerably, thereby affecting the results. For example, the present subjects had an average intake of cooked meats of 32 $g \cdot d^{-1}$. This was much lower than the estimates for Swedish (131 $g \cdot d^{-1}$) or US women (165 $g \cdot d^{-1}$) of a similar age range [18]. Perhaps even more important is the fraction of meat cooked by techniques producing HAA. The majority of women in this study (51%) indicated non-HAA-forming techniques like boiling and steaming as preferred ones to cook meat. This implies an average consumption frequency of grilled, broiled and pan-fried meats of 1.4 times per week, i.e. 73 HAA-containing meals per year. In comparison, WARD et al. [34] reported a much higher frequency of four times per week for just steaks, roasts and hamburgers.

Another noteworthy aspect of the estimation methodology is the HAA content database used in calculations. This work and several other studies used literature data, although finding levels representative for the investigated population may prove difficult [18]. Alternatively, a number of studies replicated meat cooking techniques described by participants and analysed this specifically prepared meat [24]. KEATING et al. [18] noted that such studies often reported considerably lower HAA levels and overall dietary intake. This effect occurs since an indispensable share of literature data includes experiments that maximized the production of HAA by cooking meats at high temperatures [35].

It has been observed that PhIP comprises about 70% of total HAA intake and the rest are primarily MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) [27]. Using this rule, total HAA intake (DI_{HAA}) in the present female cohort was tentatively calculated as:

$$DI_{HAA} = \left(DI_{MeIQx} + DI_{PhIP}\right) \times 1.429 \qquad (4)$$

where DI_{MeIQx} is daily intake of MeIQx, DI_{PhIP} is daily intake of PhIP and multiplication by 1.429 increases the result by 30%.

Thus, the low and high estimates of HAA intake would be 0.31 $\mu g \cdot d^{-1}$ and 4.23 $\mu g \cdot d^{-1}$ (i.e. 4.43 $ng \cdot kg^{-1}$ bw and 60.52 $ng \cdot kg^{-1}$ bw), respectively (Tab. 5). The low mean intake apparently fits better within the range of intakes reported by other researchers. Carcinogenicity of both HAA studied in this work has been proven in experimental animals, and their genotoxic action has been well documented [36]. Nevertheless, cancer typically developed at threshold levels 5×10^5- to 30×10^5-fold higher than the human dietary levels [37], which might reinforce the notion of a minor health threat that these compounds present at their usual levels in food. On the other hand, taking into account the chronic consumption pattern, genotoxic carcinogenicity also implies a need to lower the exposure and risk of cancer in high consumers and/or susceptible individuals [12].

Preliminary investigation into factors affecting HAA intake was performed to shed a light onto relationships that may need a more thorough study. For this purpose, high estimate MeIQx and PhIP intakes were calculated for subgroups of subjects divided by monthly income per household member and education level. Subgroup with the medium income range had statistically significantly higher mean intakes of each of the HAA compared to groups of both worse and better economic status. Better educational attainment was similarly connected with greater HAA exposures. Subjects with higher income and educational level included more meat into their nutrition, and this was the main factor that could explain differences between subgroups. Again, these were especially evident ($p < 0.01$) for the medium income group (42 $g \cdot d^{-1}$), compared to the high income (31 $g \cdot d^{-1}$) and low income (24 $g \cdot d^{-1}$) subjects. Neither BMI nor age of subjects were correlated to HAA intake. No consistent or significant pattern could be identified following stratification of subjects by age groups (Tab. 4).

The low estimate of daily dietary AA intake in the present population was lower, and the high estimate was higher than the estimated range of exposures in Europe (Tab. 4, 5), according to EFSA [4]. Using a tentative calculation based on AA analysis in main contributors (bread, potato crisps, fried potatoes) and their daily intakes of 100 g, BOŠNIR et al. [17] estimated that adult Croatians (70 kg) may be exposed to 1 040–1 660 $ng \cdot kg^{-1}$ bw AA. A severalfold lower mean intake was determined in adult female Polish cohort [31] and other

populations [28–30, 32, 33] (Tab. 5), which indicates that the low estimate of AA intake may also be more plausible for the population studied here. Comparison of this study to other surveys must take into account the weakness of the present design, whereas other works listed above used more elaborate methods to estimate food consumption on much larger population samples, and most of them included analysis of AA in foods. TARDIFF et al. [38] determined tolerable daily intakes of AA, based on its major toxic endpoints, neurotoxicity (40 000 ng·kg^{-1} bw) and carcinogenicity (2 600 ng·kg^{-1} bw). These values indicate a risk of carcinogenic effects in a proportion of subjects if high estimate calculations were correct (Tab. 4). This might include high consumers of foods containing intermediate AA levels. Foods contributing most to the low estimate daily intake were bread, coffee and fried potatoes, as can be seen from Fig. 1A. Fried potatoes were identified as major contributors in other studies [30, 33, 39]. On the other hand, MOJSKA et al. [31] determined that bread accounted for 49% of total AA intake. Besides methodological reasons, cultural and socio-economical characteristics of studied populations must have affected results. For example, coffee was among the biggest sources of AA in Swedish subjects [32], contributing almost two times larger quantity of AA to its total intake compared to the present cohort. Bread is a staple food in Croatia and its contribution is not surprising. Using the highest determined AA levels in bread and bakery products, the high estimate strongly increased the percentage of bread contribution (Fig. 1B) confirming its indispensable role in the diet of local population.

Comparison of mean AA intakes for subgroups divided by income and education level revealed somewhat lower intakes for the highest income and highest education attainment subgroups. The difference was only significant for the latter and was probably related to a tendency towards limiting their saccharides intake. Indeed, subjects holding a university degree consumed considerably less ($p < 0.05$) bread (156 g·d^{-1}) than subjects who finished either high school (232 g·d^{-1}) or primary school (255 g·d^{-1}). Subgroup with unfinished primary school had a mean bread intake of 368 g·d^{-1}. The same consumption pattern was determined for fried potatoes. No correlation was found between AA intake and either BMI or age of subjects. The 70–89 age group had a somewhat lower mean AA intake (Tab. 4, low estimate), but none of the differences between age group pairs were significant.

CONCLUSIONS

In this study, exposure to the investigated putative carcinogens in adult female population from Croatia was determined to be generally low. However, the results may be affected by certain limitations of the study design and a more reliable assessment should include a greater number of subjects, analysis of locally produced and/or home-cooked foods, collection of detailed information on portion size, cooking practices, especially ones known to drastically reduce formation of HAA and AA [18, 40], etc. Additional factors that should be integral parts of health risk estimation include genetic susceptibility and nutritional habits known to modulate absorption, distribution, metabolism, excretion and/or toxicity of HAA and

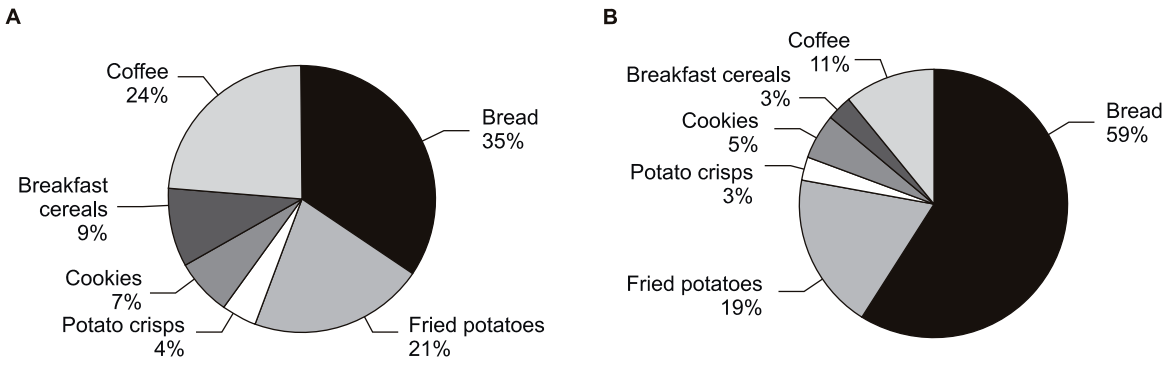

Fig. 1. Contribution of different foods to daily dietary intake of acrylamide in a female cohort from eastern Croatia.

A – low estimate, B – high estimate.
Number of participants $n = 94$.

AA in the body [41-43]. This study also provided preliminary insight into local food consumption and preparation patterns, which could be used in related research. Whilst focusing on high consumers, future studies should provide a strong basis for risk-lowering educational and other activities within the population.

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