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# Dietary acrylamide exposure from traditional food products in Lesser Poland and associated risk assessment

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**Introduction and objective.** Acrylamide (AA) is a carcinogenic and genotoxic food contaminant occurring in carbohydraterich foods produced at high cooking temperatures. The aim of the study was to determine the importance of AA exposure with respect to traditional food and to assess the associated risks.

**Materials and method.** 165 food samples were collected from local markets in Lesser Poland. The participants enrolled in the study were 500 residents: (males – 179, females – 321) who had purchased food from local markets. Exposure of the participants to AA was assessed by combining the analytical AA results with data on the individual consumption of traditional foods. Risk assessment of AA exposure from traditional foods was estimated and the margin of exposure (MOE) values were calculated.

**Results.** The highest mean AA level was measured in pretzels (92  $\mu$ g kg<sup>-1</sup>), followed by bagels (74.81  $\mu$ g kg<sup>-1</sup>) and pork paté (59.56  $\mu$ g kg<sup>-1</sup>). The average and 95th percentile values of AA exposure were 0.213 and 0.458 [ $\mu$ g kg<sup>-1</sup> body weight (BW) day<sup>-1</sup>]. The calculated values of MOE for the average [798 and 2,019 for both benchmark dose lower confidence limit (BMDL) 0.17 and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup>] and 95th percentile AA exposure values (371 and 939 for both BMDL 0.17and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup>] suggest that there is a health concern with respect to adult residents.

**Conclusions.** The results of the study confirm the general recommendation to the consumers, especially certain population groups, to eat a balanced healthy diet and to limit the amount of baked cereal products and fried products.

## Key words

acrylamide (AA); traditional food; GC-MS; dietary exposure; risk assessment

## INTRODUCTION

Food safety is a prerequisite for food and nutritional security and an area of public health action [1]. Among other hazards, acrylamide - AA (2-propeamide, CAS No. 79-06-1) - attracts a special attention. The main awareness of this chemical compound in the diet came to light when Swedish scientists discovered large amounts of AA in food products rich in starch that had been heated at high temperatures [2]. AA, therefore, is not a substance that is added to food, but is formed in food during heat processing [3]. It was shown that AA is one of the products of the Maillard reaction, a reaction between free asparagine and reducing sugars (glucose, fructose) [4, 5]. Asparagine alone may be converted thermally into AA through reactions of decarboxylation and deamination. However, the main product of the thermal decomposition of asparagine is maleimide (fast cyclization prevents the formation of AA). Nonetheless, asparagine - in the presence of reducing sugars - is able to generate AA, in addition to maleimide [6].

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In 2015, following a request from the European Commission, the European Food Safety Authority (EFSA) delivered a scientific opinion on AA in food. To-date, data from human studies, both epidemiological and on biomarkers, are still inadequate for dose-response assessment, therefore a dose-response relationship has been set based on animal results [7]. Experts have proposed two different BMDL<sub>10</sub> (lower limit on the benchmark dose for a 10% response) for AA: 0.17 mg kg<sup>-1</sup> BW day<sup>-1</sup> for neoplastic effects in mice and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup> for peripheral neuropathy in rats. The margin of exposure (MOE) for the cancer-related effects of AA, corresponding to the ratio between the  $BMDL_{10}$  and the dietary exposure of the population, ranges from 425 for average adult consumers down to 50 for high consuming toddlers; these ranges indicate a concern for public health [8]. The Commission regulation (EU) 2017/2158 of 20 November 2017 has approved AA as carcinogenic agent and established mitigation measures and benchmark levels for the reduction of the presence of AA in food [9].

Very little information on AA exposure in Poland is available. It was detected in grain coffee at Polish market. The estimated average daily intake from was 0.0023  $\mu$ g kg<sup>-1</sup> BW day<sup>-1</sup> for the entire Polish population [10]. In another study, average AA intake was 0.85  $\mu$ g kg<sup>-1</sup>BW day<sup>-1</sup>. Total dietary exposure decreased with age from 1.51  $\mu$ g kg<sup>-1</sup> BW day<sup>-1</sup> for the youngest to 0.67  $\mu$ g kg<sup>-1</sup> BW day<sup>-1</sup> for the oldest [11].

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In 2004, the Ministry of Agriculture and Rural Development in Poland introduced a law on the registration system for products of a defined geographical origin and specific traditional quality within the meaning of EU regulations. Under this law, the Ministry is responsible for receiving, evaluating and forwarding applications for the registration of designations of origin, geographical indications and traditional specialties guaranteed to the European Commission [12]. Each province has its own list of traditional products. The traditional food in Lesser Poland (southern provinces) consists mostly of various bakery products and meat and fish products, all known to be subject to AA formation during their production. Therefore, they may be potential sources of exposure to AA, considering that the inhabitants eat them almost daily throughout the year.

# OBJECTIVES

Despite the availability of previous studies on AA in some Polish food products [10, 11], no study to-date has documented AA levels in traditional food to which a large part of population is exposed. Therefore, the aim of the presented study was to determine the importance of traditional food products collected from markets in Lesser Poland (Southern Poland) causing AA exposure, and to assess the risk for the adult population (18–55 years), considering their frequency of consumption.

The required permissions for the study were obtained from the local government.

## MATERIALS AND METHOD

**Samples.** During February – April 2018, 165 samples were collected from local markets in the Lesser Poland region, and interviews conducted simultaneously with purchasers. The collected samples included the following:

- 1. sweets and snacks (15 samples of each food type, 60 total samples): cookies, cheesecakes, salty sticks and shortbread snacks (precelki Krakowskie);
- 2. bakery products (15 samples of each food type, 75 total samples): bread, a local wheat bakery product (kukiełka Lisiecka), soft pretzels (obwarzanek), bagels and pretzels;
- 3. meat and fish products (15 samples of each food type, 30 total samples): pork paté, fish preserves.

The foods were produced by a supplier responsible for producing and supplying traditional foods from a governmental list in Lesser Poland, with the exception of the salty sticks, shortbread snacks and bagels, which were produced commercially by different companies.

**Chemicals and instrumentations.** The solvents (acetonitrile and n-hexane) were of high-performance liquid chromatography (HPLC) grade purchased from Merck KGaA, Germany. Deionised water (18 M $\Omega$ ) was produced by the Milli-Q system in Millipore, USA. PSA (primary secondary amine) and C18 (octadecyl) SPE Bulk Sorbent were obtained from Agilent Technologies, USA. Acrylamide (AA), acrylamide-d<sub>3</sub> (AA-d<sub>3</sub>) (internal standard) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Sigma-Aldrich, USA.

**Preparation of reference solutions.** Stock (1 mg mL<sup>-1</sup>), intermediate (100  $\mu$ g mL<sup>-1</sup>) and working standard solutions of AA (1  $\mu$ g mL<sup>-1</sup>), and AA-d<sub>3</sub> (5  $\mu$ g mL<sup>-1</sup>) were prepared in acetonitrile.

Sample preparation procedures. Thoroughly homogenized samples were prepared by mixing equal weights of all 15 samples collected for each product. Three sub-samples of this homogenized sample were then used for AA determination. The samples were prepared using previously described methods, with a few modifications [13, 14]. The analytical protocol used in this study was previously optimised and validated in-house [15]. In brief, 1 g of the homogenized sample was accurately weighed into each of three 50-mL centrifuge tubes. Each sample was spiked with 40 µL of the AA-d, prior to the addition of 5 mL of water and 10 mL of acetonitrile (MeCN). The samples were shaken vigorously for 1 min. Next, 1 g of NaCl and 4 g MgSO, were added; samples were then again shaken vigorously for 1 min. and centrifuged (MPW 350 R Centrifuge; MPW Med. Instruments, Poland) for 15 min. at 9,000 rpm. 8 mL of the supernatant was transferred into a 15 mL PP tube, and the extracts kept in freezer at -20 °C overnight to freeze out the fat. The extracts were immediately filtrated in a freezer through a filter paper to remove precipitated co-extractives. 6 mL of each filtrate was transferred to the 15 mL PP tube containing 0.15 g of PSA sorbent, 0.3 g of C18 sorbent and 0.9 g of MgSO. The tubes were shaken for 2 min. and centrifuged for 15 min. at 10,000 rpm. A portion of the extract (2 mL) was transferred into a 4 mL screw cup vial and the extract evaporated to dryness under a stream of N2.

For derivatisation, the residues were dissolved in 500  $\mu$ L of MeCN and placed in a vial containing 50  $\mu$ L of BSTFA. The mixture was heated for 1 h at 65 °C (AccublockTM; Labnet, USA). After cooling to ambient temperature, 200  $\mu$ L of hexane were added and liquid-liquid extraction was performed for 1 min. using a vortex (MS1 Minishaker, IKA, Germany). 100  $\mu$ L of the upper hexane layer was transferred to insert and 1  $\mu$ L of extract analyzed.

Chromatographic conditions. The AA contents in the samples were determined by GC-MS. The analysis was performed using a Varian IonTrap 4000 GC/MS (Varian, Inc., USA) with a CP-8410 auto-injector (Bruker, USA) and DB-5MS column (30 m x 0.25 mm x 0.25 µm; Agilent Technologies, USA). The injector temperature was set at 270 °C, with an injection volume of 1.0 µL. The GC oven was operated with the following temperature programme: 50 °C – 3 °C min<sup>-1</sup> – 100 °C – 25 °C min<sup>-1</sup> – 250 °C (5.0 min.). The analyses were carried out with a solvent delay of 8.0 min. Helium 5.0 (Linde Gas, Poland) was used as the GC carrier gas at a flow rate of 1.0 mL min<sup>-1</sup>. The emission current of the ionisation filament was set at 15  $\mu$ A. The ion trap mass spectrometer was operated in the internal ionisation mode. The trap and the transfer line temperatures were set at 180 °C and 230 °C for analyses. Analyses were conducted in the selected ion monitoring mode (SIM) based on the use of one quantitative ion of BSTFA derivatives of AA. Confirmation ions and retention times were also used to ensure identification of the analytes, as described by [15]. Acquisition and processing data were performed using Varian Start Workstation software and NIST 2.0 library.

**Dietary AA exposure assessment.** A cross-sectional descriptive study was conducted over 3 months, from February – April 2018. 500 participants enrolled in the study: 179 males and 321 females who had purchased food on local markets.

A multistage, stratified sampling technique was used to recruit the study sample who were randomly selected. The study included local markets Lesser Poland, selected by systematic random sampling procedure. A food frequency questionnaire (FFQ) was administered to investigate the residents' consumption of food from the local markets. Traditional food available in the local markets were recorded in the FFQ. All selected participants were asked to give their BW to the nearest '1 kg'. Exposure of residents to AA was assessed by combining the analytical AA results with the data on the individual consumption of the traditional foods. Exposure as a result of traditional food was calculated using the formula:

$$E_{i} = \frac{\sum Q_{ik} \times C_{ik}}{BW_{i}}$$

where  $E_i$  is the dietary AA exposure of the participants ( $\mu g k g^{-1} BW da y^{-1}$ ),  $Q_{ik}$  is the amount of food item k consumed by a participant during one day,  $C_{ik}$  is the AA concentration in food item k ( $\mu g k g^{-1}$ ),  $BW_i$  is the BW of the participant i (kg), and  $\Sigma$  denotes the sum of all food items consumed by the participant i among the traditional foods.

**Risk assessment of AA exposure from traditional food.** For assessment of the risk, the margin of exposure (MOE) values were calculated by comparing the mean and 95th percentile values of AA exposure against  $BMDL_{10}$  values (0.17 mg kg<sup>-1</sup> BW day<sup>-1</sup> for neoplastic effects, and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup> defined for peripheral neuropathy) [7, 8].

**Statistical analysis**. The obtained results were calculated using the MS Excel computer programme, and evaluated using Statistica ver. 12 (Statsoft, Inc.). The AA contamination of the traditional foods, the dietary AA exposure of the participants and selected percentiles (P10th, P50th and P95th) of the studied groups were calculated. The dietary intake of AA did not follow a normal distribution; therefore, a Mann–Whitney U-test was used to determine the significance of the difference in AA exposure between males and females.

### RESULTS

AA in traditional food. Among the 11 investigated traditional products, the highest mean AA levels were found in pretzels (92  $\mu$ gkg<sup>-1</sup>) and bagels (74.81  $\mu$ g kg<sup>-1</sup>) (Tab. 1). The AA levels in the traditional bread offered in Lesser Poland were found to be 29.74  $\mu$ gkg<sup>-1</sup>.

**Exposure assessment.** Table 2 summarizes the characteristics of AA exposure caused by traditional food. The overall mean and 95th percentile values for the AA exposure of participants were found to be 0.213 and 0.458  $\mu$ g kg<sup>-1</sup> BW day<sup>-1</sup>, respectively.

Dietary exposure was found to be higher in the younger (18– 36 years) residents compared to older residents (37–54 years and 55+ years). Average AA exposure decreased significantly with increasing age (P<0.05), from 0.32  $\mu$ g kg<sup>-1</sup>BW day<sup>-1</sup> in

	N	Concentration (µg kg )						
Food groups	Number of samples	Mean±SD	Minimum concentration	Maximum concentratior				
Sweets and snacks								
Cookies	15	0.00±0.00	0.00	0.00				
Cheesecakes	15	19.46±0.29	19.0	20.1				
Salty sticks	15	44.55±11.66	23.3	65.0				
Precelki Krakowskieª	15	47.14±1.96	44.0	50.5				
Bakery products								
Bread	15	29.74±24.72	0.00	77.7				
Kukiełka Lisiecka <sup>ь</sup>	15	44.76±3.91	37.6	50.5				
Obwarzanek	15	44.93±29.51	0.00	100				
Bagels	15	74.81±33.7	34.2	143				
Pretzels	15	92±15.72	69.3	124				
Meat and fish prod	ucts							
Pork pate	15	59.56±16.03	36.5	83.0				
Fried fish preserves	15	22.7±8.3	9.11	34.1				

Table 1. Acrylamide levels (µg kg<sup>-1</sup>) in traditional food in Lesser Poland

Concentration (ug kg<sup>-1</sup>)

the youngest group to 0.083  $\mu$ g kg<sup>-1</sup>BW day<sup>-1</sup> in the oldest group. The 95th percentile values of AA intake also decreased with increasing age, from 0.51  $\mu$ g kg<sup>-1</sup>BW day<sup>-1</sup> in 18–36-yearold residents to 0.31  $\mu$ g kg<sup>-1</sup>BW day<sup>-1</sup> and 0.221  $\mu$ gkg<sup>-1</sup>BW day<sup>-1</sup> in 37–54 and 55+ year-old-residents, respectively. The same trend was observed in the P10th, P50th and average exposure values (Tab. 2). No significant difference was found between males and females in exposure to AA from the consumption of traditional food (Tab. 2).

The contribution of each of the traditional products to the mean dietary exposure of each group to AA is shown in Table 3. Bread, cookies, pork paté, cheesecakes, salty sticks, bagels and soft pretzels represent the major sources of AA exposure among the traditional foods in the youngest participants. These 7 products represent the majority (78.1%) of the total AA exposure of 18-36-year-old residents. Bread, pork paté, cookies, cheesecakes, soft pretzels, pretzels and fish preserves represent the major sources of AA exposure among the traditional foods in middle group. These seven products represent the majority (80%) of the total AA exposure of 37-54 years old residents. Bread, obwarzanek, pork pate, fish preserves, shortbread snacks, bagels and pretzels represent the major sources of AA exposure among the traditional foods in the oldest participants. These 7 products represent the majority (76.8%) of the total AA exposure of 55+ yearold residents.

**Risk assessment**. Given that AA is a possible genotoxic carcinogen, the MOE approach may provide an idea of the risks associated with its presence in food [16, 17]. The MOE approach remains the most common method of risk characterization with respect to AA and all other food chemicals, despite some uncertainties derived from the use of data from rodents to assess the dose-response curve, and the lack of human external or internal exposure studies to confirm its validity [18].

#### Table 2. Mean acrylamide exposure (µg kg<sup>-1</sup> BW day<sup>-1</sup>) from traditional foods among males and females of different age groups

Maximum 0.572 0.549 0.572 0.389 0.340	Significance between males and females (Mann–Whitney U-test) 0.529 <sup>b</sup> 0.757	Significance between age group: (Kruskal–Wallis test) 0.056 <sup>b</sup>
0.549 0.572 0.389		0.056 <sup>b</sup>
0.572 0.389	0.757	
0.389	0.757	
	0.757	
0.340		
0.389		
0.287	0.834	
0.269		
0.287		
0.572	1.000	
0.549		
	0.572 0.549	0.572 1.000

<sup>a</sup> 95th percentile (high exposure); <sup>b</sup> p-value (P), the differences are significant if P < 0.05.

#### Table 3. Contribution of each traditional food to the mean acrylamide exposure (µg kg<sup>-1</sup> BW day<sup>-1</sup>) among residents

	Age group (18–36 years)						Age group (37–54 years)				Age group (55+ years)				
Percentiles <sup>a</sup>					Percentiles				Percentiles						
Food item	Contribution					Contribution				Contribution					
	Mean	P10th	P50th	P95th	%	Mean	P10th	P50th	P95th	%	Mean	P10th	P50th	P95th	%
Cookies	0.040	0.010	0.023	0.122	12.5	0.020	0.006	0.017	0.043	10	0.005	0.002	0.004	0.009	6.1
Cheesecakes	0.030	0.005	0.021	0.088	9.4	0.020	0.005	0.017	0.038	10	0.005	0.002	0.004	0.012	6.1
Salty sticks	0.030	0.010	0.021	0.087	9.4	0.010	0.003	0.009	0.021	5	0.004	0.002	0004	0.008	4.9
Precelki Krakowskie	0.020	0.000	0.013	0.075	6.3	0.010	0.002	0.009	0.022	5	0.008	0.002	0.008	0.017	9.8
Bread	0.050	0.010	0.028	0.136	15.5	0.030	0.008	0.021	0.088	15	0.010	0.003	0.008	0.019	12.2
Kukiełka Lisiecka	0.010	0.000	0.009	0.216	3.0	0.010	0.003	0.009	0.020	5	0.005	0.002	0.004	0.012	6.1
Obwarzanek	0.020	0.005	0.017	0.043	6.3	0.020	0.005	0.017	0.054	10	0.010	0.003	0.008	0.018	12.2
Bagels	0.030	0.005	0.021	0.090	9.4	0.010	0.003	0.008	0.021	5	0.008	0.003	0.005	0.018	9.8
Pretzels	0.030	0.010	0.021	0.078	9.4	0.020	0.004	0.015	0.062	10	0.008	0.003	0.008	0.017	9.8
Pork pate	0.040	0.011	0.027	0.099	12.5	0.030	0.006	0.020	0.090	15	0.010	0.003	0.009	0.021	12.2
Fish preserves	0.020	0.005	0.017	0.044	6.3	0.020	0.004	0.017	0.054	10	0.009	0.003	0.008	0.018	10.8
Total	0.320	0.121	0.321	0.510	100	0.200	0.110	0.200	0.310	100	0.082	0.028	0.057	0.221	100
Total	0.320	0.121	0.321	0.510	100	0.200	0.110	0.200	0.310	100	0.082	0.028	0.057	0.	.221

<sup>a</sup> - Percentiles (P10th, P50th and P95th) for each food were calculated for consumers of each traditional food only. However, the total corresponds to all respondents

Table 4. Estimation of margin of exposure (MOE) for	r mean and 95th percentile of acrylamide dietary exposure

	18–3	6 years	37–54	4 years	55+	years	Total (18–55+ years)	
Age group	Mean	P95th <sup>a</sup>	Mean	P95th	Mean	P95th	Mean	P95th
Dietary exposure (μg kg <sup>-1</sup> BW day <sup>-1</sup> )	0.32	0.51	0.20	0.31	0.0082	0.221	0.213	0.458
$MOE (BMDL_{10} = 0.17 \text{ mg kg}^{-1} BW \text{ day}^{-1})^{a}$	531	333	850	548	2073	769	798	371
$MOE (BMDL_{10} = 0.43 \text{ mg kg}^{-1} BW \text{ day}^{-1})^{\text{b}}$	1344	843	2150	1387	5244	1946	2019	939

MOEs, margins of exposure; BMDL, viewer limit on the benchmark dose for a 10% response.<sup>a</sup> BMDL, defined for neoplastic effects (EFSA, 2015), <sup>b</sup> BMDL, defined for peripheral neuropathy (EFSA, 2015).

The MOE values calculated for the overall mean AA exposure were 798 and 2,019 for the both  $BMDL_{10}$  values (0.17 and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively) (Tab. 4). For the different age groups, the MOE values calculated for mean AA exposure increased with age from 531 to 2,073 for the  $BMDL_{10}$  value of 0.17 mg kg<sup>-1</sup> BW day<sup>-1</sup>, and from 1,344 to 5,244 for 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup>. The same trend was also observed for the 95th percentile of exposure, for which the

values of MOE increased with age from 333 to 769, and from 843 to 1946 for the both  $BMDL_{10}$  values, respectively.

For the high-consumption group, the AA exposure exceeded the average exposure (mean exposure 0.458-0.572 µg kg<sup>-1</sup> BW day<sup>-1</sup>); therefore, the calculated MOE for the high-consumption group are lower (297-371 and 752-939 for BMDL<sub>10</sub> values of 0.17 and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively).

## DISCUSSION

AA in traditional food. Traditional foods are often associated with specific ingredients and production technology, and thus with higher quality and safety [19]. The types of foods sold in local markets in Lesser Poland are various bakery products and meat and fish products which have been identified in many previous studies as being important sources of AA. Therefore, the AA contamination of the traditional foods and the dietary AA exposure should attract a special attention.

Most traditional food (except from cookies) offered in the markets of Lesser Poland were found to contain levels of AA higher than the LOD and LOQ. This is because all these products are carbohydrate-rich foods that are prepared at high temperatures, which leads to AA formation. It is also important to note that suppliers use almost the same ingredients and cooking conditions for each traditional product.

The levels of AA detected in this study for bagels  $(74.81 \ \mu g \ kg^{-1})$  and pretzels  $(92 \ \mu g \ kg^{-1})$  were consistent with the levels measured in Latvia (39-588  $\mu$ g kg<sup>-1</sup>) [20], and with results for pretzels in Canada (71 µg kg<sup>-1</sup>) [21]. The AA levels in traditional bread (29.74 µg kg<sup>-1</sup>) offered in Lesser Poland were found to be nearing the levels measured in similar products in a Latvian total diet study (12-52 µg kg<sup>-1</sup>). In general, AA formation in bakery products is highly variable, ranging from non-detectable to approximately 90 µg kg<sup>-1</sup>. This is attributed to the type of cereals used in flour production, as well as to the baking process time and temperature used to develop the characteristic features [22]. Furthermore, the variations in AA concentration among samples of the same type, or from the same group of foods that have appeared in many studies, have been attributed to variations in heating temperature and time, different types and amounts of carbohydrate, different laver thicknesses of the products, the presence of different amino acids and other additives, and different types of fillings used in some products [23, 24, 25].

Exposure assessment. The overall mean and 95th percentile values for the AA exposure from the consumption of traditional foods during the day were found to be 0.213 and 0.458 µg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively. This intake is similar to or lower than the estimated total daily AA intake in several other studies. For example, the estimated dietary intake among a Chinese population given as the mean and the 95th percentile (P95th) were 0.286 and 0.490 µg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively [26]. In Japan, average dietary exposure to AA was estimated as 0.147-0.154 µg kg<sup>-1</sup> BW day<sup>-1</sup> (95th percentile, 0.226-0.261 µg kg<sup>-1</sup>BW day<sup>-1</sup>) [27]. In France, the average dietary intakes have been found to be 0.45 and 0.69  $\mu g\,kg^{\mbox{--}1}\,BW\,day^{\mbox{--}1}$  for adults and children, respectively, and the 95th percentile values are 1.71 and 1.8 µg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively [28]. According to the JECFA data, the estimates of mean AA intake among 17 countries range from 0.3–2.0 µg kg<sup>-1</sup> BW day<sup>-1</sup> [29]. The JECFA has also estimated that the mean dietary exposure to AA in the general adult population to be in the range 0.2-1.0  $\mu$ g kg<sup>-1</sup> BW day<sup>-1</sup>, with the highest intake estimated to be 0.6-1.8 μg kg<sup>-1</sup> BW day<sup>-1</sup> [30].

In the presented study, average AA exposure decreased significantly with increasing age (P<0.05). The 95th percentile values of AA intake also decreased with increasing age. The same trend was observed in the P10th, P50th and average exposure values (Tab. 2). This finding is similar to those obtained in several studies, including another study from

southern Poland, where AA exposure was found to decrease from  $1.51 \ \mu g \ kg^{-1} BW \ day^{-1}$  in the youngest group (6–12 years) to 0.67  $\ \mu g \ kg^{-1} BW \ day^{-1}$  in the oldest group (42–60 years) [11].

Bread, cookies, pork paté, cheesecakes, salty sticks, bagels, shortbread snacks, soft pretzels, pretzels, and fish preserves represent the major sources of AA exposure among the traditional foods for the 3 age groups. The high contribution of these products can be attributed to their high levels of AA contamination or their high consumption rates, or both. The differences between age groups in terms of the foods that contribute most significantly to total dietary AA exposure can be primarily attributed to differences in consumption habits. In Poland, bakery products are the main source of AA because of the high consumption of bread by the Polish population [11]. Similarly, in Germany, where 83 kg of bread is consumed per inhabitant per year, a significant proportion of the total intake of AA originates from the ingestion of bread and other bakery products (18-46%) [31]. For most other countries, the foods that represent the largest contributions to dietary AA exposure are French fries, potato chips, breadstuffs, and pastries and cookies (10-60%, 10-22%, 13-34% and 10-15%, respectively) [11, 16, 32]. French fries and other potato products are the most important contributors to AA intake in many countries, such as in France (45% for adults and 61% for children) [28] and the USA (where potato chips are the main contributor) [29] because of their particularly high levels of both consumption and AA contamination. Fortunately, the consolidated list of traditional products issued by the Ministry of Agriculture and Rural Development of Poland do not include French fries and potato chips as part of the food products or the dishes; otherwise, significantly higher levels of AA intake might be expected.

Risk assessment. The margins of exposure (MOE) values calculated for the overall mean AA exposure were 798 and 2,019 for the both  $BMDL_{10}$  values (0.17 and 0.43 mg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively) (Tab. 4). The obtained MOE values from the current study are much higher than those suggested by the EFSA (50-425) and JECFA (45-310), and indicate an AA health concern. The EFSA/WHO considers a public health issue to exist, requiring efforts to reduce exposure when the MOE value is lower than 10,000 based on a given BMDL<sub>10</sub> [33]. The low MOE values obtained for exposure to traditional food indicate that AA is an important issue of concern for the inhabitants of Lesser Poland with respect to the EFSA opinion. This means that the general exposure levels of AA will be higher and potential risks will increase, especially with the high rate of consumption of bakery products by the Polish population. The mean intake of bakery products by the Polish population is 56.5 kg per year [34]. It was also found that the mean AA intake from bakery products for 20-30-year-olds, 31-41-year-olds and 42-60-year-old adults were 0.33 µg kg<sup>-1</sup> BW day<sup>-1</sup>, 0.31 µg kg<sup>-1</sup> BW day<sup>-1</sup> and 0.25 µg kg<sup>-1</sup> BW day<sup>-1</sup>, respectively [11].

Based on all the above data, certain actions should be taken to reduce AA exposure from traditional foods. Examples of such actions already taken include mitigation studies conducted in Switzerland, Belgium and Lithuania, as well as a dietary modification study conducted in Finland, which led to significant decreases in these countries in AA content in many products, including potato products, bread and rolls, breakfast cereals, chocolate and biscuits, [17, 35, 36, 37]. Recently, guidelines for manufacturers to reduce AA in foods

have been established by the EU Commission in collaboration with the European Environment Agency. Detailed guidelines for reducing AA formation in all steps of the production process, from raw materials to the final processed products, have been formulated [9].

## CONCLUSIONS

The products which contained the highest level of AA were pretzels, bagels and pork paté. The calculated MOE for the average and 95th percentile AA exposure values suggest that there is a health concern with respect to adult inhabitants, and this age group should limit the consumption of these products.

The results of this study also confirm the general recommendation to the consumer, especially certain population groups, to eat a balanced healthy diet and to limit the amount of baked cereal products and fried products.

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