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Assessment of dietary acrylamide exposure in children attending Spanish school canteens using the duplicate diet method

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ABSTRACT

Acrylamide is a carcinogenic chemical contaminant formed in heat-treated foods. In this study, a duplicate diet method was used to evaluate the acrylamide content of foods/meals served at breakfast and lunch in two Spanish school canteens. The dietary acrylamide intake in students was estimated within lower bound (LB) and upper bound (UB) scenarios. Biscuits exhibited the highest acrylamide values, exceeding the benchmark level established by the European Regulation 2017/2158 (350 μg/kg), followed by main courses such as stews, side dishes and bread. In the LB scenario, breakfasts accounted for the major contributors to the daily acrylamide intake (73.3%). However, lunches were the main responsible in the UB scenario (65.4%). Acrylamide exposure was estimated for three age ranges: 3-5y pre-school children (0.59 and 0.92 μg/kg body weight/day), 6-9y children (0.43 and 0.67 μg/kg body weight/day) and 10-12y early adolescents (0.28 and 0.44 μg/kg body weight/day). Margins of exposure for neoplastic effects ranged between 144 and 1026, which is below the reference of 10,000, indicating a health concern. The findings reveal that any diet, even one that is balanced, varied and contains foods low in acrylamide, involves an additive exposure to the contaminant that should be considered when conducting acrylamide exposure risk assessments.

1. Introduction

School canteens play a vital role in providing essential services to Spanish children throughout their schooling, especially during the early stages. In the last two decades, there has been a significant increase in the number of children and adolescents who consume their main meal of the day outside of their homes due to changes in family and social dynamics. Specifically in Spain, 36.4% of early childhood education students and 29.4% of primary school students engage in this activity (Delcampoalcole, 2018). Consequently, school meals contribute significantly to childrens' overall diet. In most cases, school canteens lack the ability to prepare their menus on-site. Instead, catering companies often provide prepared menus, with food being cooked in a central kitchen and then transported to the school for immediate consumption. Despite the efforts of health authorities in recent years to promote the improvement of the nutritional profile of school menus by limiting the presence of sugary and fatty foods and increasing the provision of vegetables and fruits to safeguard the health and well-being of schoolchildren (AESAN, 2010), processed foods that undergo cooking techniques such as frying, roasting or baking are prevalent.

Consumption of processed foods exposes children to the so-called chemical process contaminants, which are compounds naturally formed during the cooking process (Murkovic and Pedreschi, 2017). One of these contaminants is acrylamide, a compound generated during the Maillard reaction between the free amino acid asparagine and carbonyl compounds, mainly reducing sugars, at temperatures above 120 °C and in low moisture conditions. Therefore, acrylamide is commonly found in foods that have undergone baking, frying or roasting processes (Stadler et al., 2002). Acrylamide has been classified as probably carcinogenic for humans by the International Agency for Research on Cancer (IARC, 1994). To address concerns about its potential toxicity, some countries and organisations have established guidelines or regulations for acceptable acrylamide levels in foods (FAO, 2009; EC, 2017). Hence, it becomes essential not only to assess the impact of cooking practices on acrylamide formation, but also to consider the overall eating patterns that contribute to daily exposure to this contaminant. This issue is particularly concerning for public health, with a specific focus on children due to their dietary habits and vulnerability arising from their smaller body weight. This vulnerability can lead to a higher relative exposure to contaminants present in foods (EFSA, 2015a).

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According to existing literature, various methods have been employed to assess dietary acrylamide exposure, involving different calculation approaches (probabilistic, deterministic), consumption estimates (24-h recall, 2- and 7-day dietary records, food frequency questionnaires, etc.) and reference levels of acrylamide in food. These methods have been applied to study both the general population and specific groups (Cressey et al., 2012; Claeys et al., 2016; Nematollahi et al., 2020; González et al., 2022). However, many of these studies have certain limitations. One notable limitation is the use of mean values to describe the presence of acrylamide in groups of processed foods, which are then combined with food consumption tables that do not account for variations in cooking methods. Additionally, the use of general databases for acrylamide occurrence, such as those provided by the European Food Safety Authority (EFSA), can lead to inaccuracies in exposure rate calculations. These issues hinder the establishment of robust and reliable conclusions, limiting the ability of national and regional institutions to effectively monitor acrylamide exposure and make progress in this area. Furthermore, dietary recall methods present methodological challenges. Participants tend to under-report intake, especially for foods considered unhealthy, which can introduce recall bias and difficulties in classifying products within a food category (Thompson and Subar, 2017). To improve exposure assessment and obtain more accurate data, EFSA recommends conducting duplicate diet studies to estimate the real dietary intake of contaminants and evaluate food safety risks and potential adverse effects on human health (EFSA, 2015a). However, there have been very few research studies employing duplicate diet methods to assess real exposure to acrylamide in the diet, with no such research existing in the Spanish context. In light of this, the aim of the present study was to assess acrylamide exposure in Spanish school children who eat at school canteens using the duplicate diet method recommended by EFSA. This study will provide estimations of the risk associated with such exposure in Spanish school-age children for the first time, offering valuable insights into the potential health implications.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals, solvents and reagents used for acrylamide determination were of analytical grade. Acrylamide standard (99%), potassium hexacyanoferrate (II) trihydrate (98%, Carrez-I) and zinc acetate dihydrate (>99%, Carrez-II) were purchased from Sigma (St. Louis, MO, USA). Formic acid (98%), methanol (99.5%) and hexane were obtained from Panreac (Barcelona, Spain). [$^{13}C_3$]-acrylamide (99%) was acquired from Cambridge Isotope Laboratories (Andover, MA, USA). Oasis-HLB cartridges (30 mg, 1 mL) were obtained from Waters (Milford, MA, USA) and Cellulose syringe filter units (0.22 and 0.45 μm) were acquired from Análisis Vínicos (Tomelloso, Ciudad Real, Spain). Deionised water from a Milli-Q Integral 5 water purification system (Millipore, Billerica, MA, USA) was used to prepare all solutions.

2.2. Study design and sample collection

Two primary schools located in different regions of Spain, one in Madrid (centre) and the other in Castilla la Mancha (mid-east) were recruited for the study. The school canteens usually prepare daily meals for approximately 60 primary school students aged 3–12 years. They follow a 4-week menu rotation that aligns with the recommendations for a healthy and balanced diet to prevent childhood obesity, as outlined in the Spanish Strategy for Nutrition, Physical Activity and the Prevention of Obesity promoted by the Spanish Council on Food Safety and Nutrition (BOE, 17/2011 Law). The present study took place over two periods, between February 2020 and May 2021. Both schools provided meals cooked in their own kitchens for a week in February and another week in May with the aim of collecting menus corresponding to winter and summer seasons, respectively. Each school canteen participated in a two-week trial (Fig. 1), during which all foods and meals served for breakfast and lunch from Monday to Friday were collected using the duplicate diet method (Shim et al., 2014; Porca Fernández et al., 2016). Table 1 provides additional details related to the study. Breakfasts were the same over the two weeks in each school. A total of 167 different meals were collected, consisting of 70 individual foods from breakfasts (41.9%) and 97 individual foods/meals from lunches (58.1%). After collection, the samples were transported to the laboratory using a refrigerated courier service. In the laboratory, the samples were coded, photographed and processed. To provide a comprehensive description of the foods, each meal was classified according to FoodEx 2 classifications provided by EFSA (EFSA, 2011, 2015b). These classifications use pre-established facets and descriptors to categorise the foods. All portions of the collected meals were edible, with the exception of the potato and pork ribs stew (282 g), chicken drumsticks (209 g) and breaded horse mackerel fillets (112 g), where the bones were removed. The edible portions were weighed and homogenised using a hand blender (Taurus, vital CM, Spain). Aliquots of each meal were then stored at −20 °C until further analysis.

2.3. Acrylamide determination by LC-ESI-MS/MS

The acrylamide content of meals compounding breakfast and lunch was determined following the method described by González-Mulero et al. (2021). Two g of ground sample was weighed in polypropylene centrifugal tubes and mixed with 37.6 mL of Milli-Q water. Next, 4 mL of hexane was added to the tubes. Samples were spiked with 400 μL of a 5 μg/mL [$^{13}C_3$]-acrylamide methanolic solution as internal standard and homogenised (Ultra Turrax, IKA, Mod-T10 basic, Bohn, Germany) for 15 min. Then, 250 μL of both Carrez I (15 g of potassium ferrocyanide/100 mL of water) and Carrez II solutions (30 g of zinc acetate/100 mL of water) were added and centrifuged at 4 °C, 9000×*g* for 10 min. Following this, the hexane layer was removed and supernatants were filtered through a 0.22 μm cellulose filter. Clear supernatants were cleaned using an Oasis-HLB cartridge, previously conditioned with 1 mL of methanol and 1 mL of distilled water, at a flow rate of 2 mL/min. The first drops were discharged and the remainder was collected using an amberlite LC-MS vial.

Acrylamide from sample extracts and calibration standards was determined by an Agilent 1200 liquid chromatograph coupled to an Agilent Triple Quadrupole MS detector (Agilent Technologies, Palo Alto, CA, USA). An Inertsil ODS-3 column was used (250 × 4.6 mm, 5 μm; GL Sciences Inc., Tokyo, Japan) at 30 °C to achieve analytical separation. Isocratic elution was employed with a mobile phase of formic acid in water (0.2 mL/100 mL) at a flow rate of 0.4 mL/min. The injection volume was 10 μL with the needle set at 1.0 kV. Electrospray ionisation was used in the positive mode. Acrylamide was eluted for 6.1 min under these chromatographic conditions. Nitrogen was used as the nebulizer gas (12.0 L/min) and the source temperature was set at 350 °C.

For acrylamide and [$^{13}C_3$]-acrylamide, signals at *m/z* 72.1–*m/z* 55.1 and *m/z* 75.1–*m/z* 58.1 were isolated, respectively. Fragmentation was set at 76 V and collision energy was set at 8 V for the transition *m/z* 72.1 > *m/z* 55.1 and at 9 V for *m/z* 75.1 > *m/z* 58.1. Masses were recorded using multiple reactions monitoring (MRM). The recovery rate of samples when spiked with acrylamide ranged between 92 and 103%. Relative standard deviations (RSDs) for all determinations in the analyses were less than 10%. Samples were analysed at least by duplicate. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated by injecting lower concentrations of standards. A concentration determined to have a signal-to-noise ratio of 3 was assigned to LOD (4.5 μg/kg), whilst a concentration determined to have a signal-to-noise ratio of 10 was assigned to LOQ (15 μg/kg). Precision, repeatability and reproducibility of the analytical method were evaluated by analysing different samples on the same day (precision), outcomes produced by different operators (repeatability) and outcomes produced

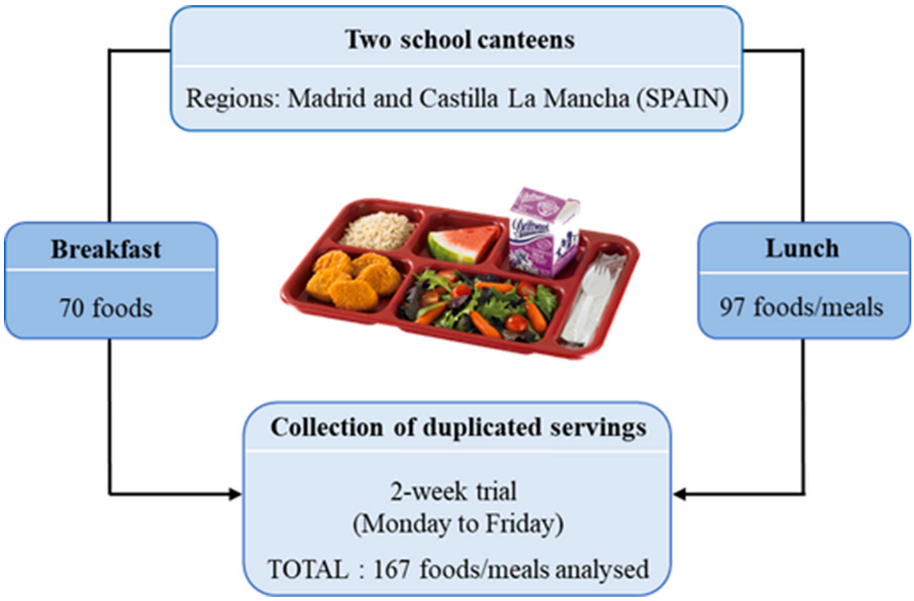

Fig. 1. Design of the duplicate diet study in school canteens.

Table 1
Description of the menus provided by the school canteens.

	Menu	Monday	Tuesday	Wednesday	Thursday	Friday
School 1	**Breakfast**	Milk	Milk	Milk	Milk	Milk
		Fruit juice	Fruit juice	Fruit juice	Fruit juice	Fruit juice
		Biscuits	Cornflakes	Chocolate puffed rice breakfast cereals	Cornflakes	Biscuits
	Lunch week 1	Spaghetti bolognese	Beans with sausage stew	'A banda' rice[a]	Sauteed peas with egg and ham	Lean pork and potato stew
		Battered squid rings	Beef burger	French omelette	Roasted chicken drumsticks	Pomfret in tomato sauce
		Mixed salad	Pilau rice[a]	Mixed salad	French fries	Mixed salad
		Pear	Apple	Orange	Yoghurt	Banana
		Bread	Bread	Bread	Bread	Bread
	Lunch week 2	Noodles with red pepper and garlic	Zucchini cream	Lentils stew	Mixed paella	Traditional chickpea-based stew prepared with meat and vegetables
		Breaded monkfish	Grilled pork loin with sauteed vegetables	Tuna in tomato sauce	French omelette	Peach in syrup
		Mixed salad	Mixed salad	Sauteed pasta	Mixed salad	Bread
		Pear	Orange	Apple	Yoghurt	
		Bread	Bread	Bread	Bread	
School 2	**Breakfast**	Milk	Milk	Milk	Milk	Milk
		Soluble cocoa	Soluble cocoa	Soluble cocoa	Soluble cocoa	Soluble cocoa
		Fruit	Fruit	Fruit	Fruit	Fruit
		Biscuits	Bagel	Puff pastry biscuits	Muffin	Whole wheat biscuit
	Lunch week 1	Pasta with sausage, cheese and tomato sauce	Sauteed chard with ham	Potato and pork ribs stew	Carrot cream	Traditional chickpea-based stew prepared with meat and vegetables
		Baked cod	Turkey stew	Breaded horse mackerel fillets	Battered chicken cutlets	Salad
		Mixed salad	White rice	Mixed salad	Mixed salad	Melon
		Apple	Watermelon	Banana	Yoghurt	Bread
		Bread	Bread	Bread	Bread	
	Lunch week 2	Sauteed peas with bacon	Pasta salad	Brothy rice with chicken and vegetables	Sauteed of boiled chard and potato	White beans with sausage stew
		Baked pomfret in tomato sauce	Breaded burger	Breaded hake	Roasted chicken drumsticks	Tuna omelette
		Mixed salad	French fries	Mixed salad	Mixed salad	Mixed salad
		Nectarine	Apple	Melon	Yoghurt	Watermelon
		Bread	Bread	Bread	Bread	Bread

[a] Pilau rice: typical meal of Indian cuisine consisting on stir-fried rice with vegetables, meat and spices. 'A banda' rice: typical meal consisting in rice cooked in fish broth.

on different days (reproducibility).

The accuracy of findings was examined by performing proficiency tests launched by the Food Analysis Performance Assessment Scheme (FAPAS) program. The latest results for the food matrices provided for FAPAS pertained to crispbread (test 30118, January 2022), coffee (test 30117, December 2021) and potato crisps (test 30120, April 2022) yielding z-scores of −0.1, 0.1 and 0.4, respectively.

2.4. Estimation of acrylamide exposure

A deterministic approach was employed to calculate the acrylamide exposure of children through the consumption of foods and meals provided by the school canteens. The individual contribution of the acrylamide from each meal was calculated by combining its acrylamide content and the weight of the edible portion of the serving. Processed foods with acrylamide concentrations below the LOQ (<15 μg/kg) were replaced by zero and LOQ values, in lower bound (LB) and in upper bound (UB) scenarios, respectively, as suggested in literature for studies in the field of food safety (Branciari et al., 2020; EFSA, 2015a). Dietary acrylamide exposure in schoolchildren was calculated by summing the acrylamide contributions of all the foods/meals consumed during each single day at breakfast and lunch during the 2-weeks of the study, and then dividing this total by the children body weight (bw) (Equation (1)).

$$\textbf{Estimated dietary exposure} = \Sigma \frac{\text{Food consumption}\left(\frac{\text{kg}}{\text{day}}\right) \text{x Acrylamide content of food} \left(\frac{\mu\text{g}}{\text{kg}}\right)}{\text{Body weight (kg)}} \qquad \text{Eq. 1}$$

Three different mean values of bw were used for this estimation, in accordance with age-associated data (Fernández et al., 2011) and age classifications of the Spanish educational system: 1) Pre-school children aged 3–5 years (mean bw = 19 kg); 2) Primary school children aged 6–9 years (mean bw = 27 kg); 3) Primary school early adolescents aged 10–12 years (mean bw = 40 kg). Finally, average daily exposure was calculated for a 10-day period during which the regular canteen menu was served at each school. Results were expressed as μg/kg bw per day.

An acrylamide exposure risk assessment was performed by computing the margin of exposure (MOE) for both neurotoxic and neoplastic effects in LB and UB scenarios, according to the following formula:

$$\textbf{MOE} = \frac{\text{BMDL}_{10}(\mu\text{g/kg bw per day})}{\text{Estimated dietary exposure } (\mu\text{g/kg bw per day})} \qquad \text{Eq. 2}$$

For neurotoxic effects, a benchmark dose lower confidence limit ($BMDL_{10}$) for neurotoxicity of 430 μg/kg bw per day, previously calculated in rats was used. MOEs lower than 125 may indicate a human health concern. For carcinogenic effects, a $BMDL_{10}$ of 170 μg/kg bw per day, previously calculated in Harderian gland tumours in male mice was used. In this case, risk is indicated with MOEs lower than 10,000 (EFSA, 2015a).

2.5. Statistical analysis

All statistical analyses were performed using Statgraphics Centurion XVI.I. The data are expressed as means and standard deviations (SD). One-way ANOVA, followed by Tukey HSD test, was used to identify the overall significance of differences in acrylamide exposure among individuals belonging to the three examined age ranges. Student's t-test was also used to analyse significant differences between the two participating schools. This analysis compared both overall acrylamide intake and acrylamide exposure according to age. Variance homogeneity was determined by the Levene's test. All statistical parameters were evaluated assuming a significance level of $p < 0.05$.

3. Results and discussion

3.1. Characterisation of menus provided in school canteens

Table 1 presents the individual foods and meals comprising the menus served at school canteens during the two-week trial. The composition of these menus aligns with the recommendations outlined in various consensus documents on nutrition in schools published in Spain (AESAN, 2008, 2010; Agencia de salud pública de Cataluña, 2020). For breakfast, the guidelines suggest including a dairy product, a cereal-based food (breakfast cereal or biscuits), fruit and other items like olive oil (maximum twice a week) and bakery products or confectionery (maximum once a week). For the lunch menu, the recommendations advise incorporating different proportions of vegetables, starchy foods, proteins, fruit and virgin olive oil. It is also recommended to use various culinary techniques throughout the week (i.e. boiling, frying, roasting, baking). A typical lunch should consist of a main course based on rice (once a week), pasta (once a week), legumes (once-twice a week) or vegetables (once-twice a week), including potatoes. The second course should feature a protein source like meat (once-three times a week), fish (once-three times a week) or eggs (maximum twice a week). These should be accompanied by different salads (three-four times a week) and other side dishes (potatoes, vegetables, legumes, etc., once-twice a week). Desserts should primary consist of fresh fruit most days of the week, with occasional alternatives like yoghurt, fresh cheese or fruit juices. Moreover, one portion of bread is allowed every day. It is essential to limit the serving of breaded and fried products, which should not exceed twice a week as a second course or once a week as a side dish. The school canteen menus analysed in the present study closely adhered to these guidelines, maintaining a similar distribution of foods and meals for both breakfast and lunch. This approach ensured the recommended weekly frequency of servings for all food groups and applied various cooking techniques over the course of a week.

3.2. Acrylamide content of foods and meals

The acrylamide content of meals served by school canteens for both breakfast and lunch is summarised in Table 2. Meanwhile, Table 3 shows processed meals with acrylamide levels falling between LOD and LOQ, for which LOQ value (15 μg/kg) was assigned in the UB scenario. Among the 167 foods/meals analysed, only 21% (36 samples) had acrylamide levels above the LOQ, with 16 samples from breakfast and 20 samples from lunch. This indicates that approximately 21% of samples from both breakfast and lunch contained measurable levels of the contaminant. The highest concentrations of acrylamide were found in biscuits, with mean values ranging from 646 μg/kg in school 1 to 680 μg/kg in school 2. All biscuits, except for whole wheat biscuits, almost doubled the benchmark levels (BL) established by the European Regulation 2017/2158 for these foods (350 μg/kg) (EC, 2017). This finding is consistent with previous reports on other branded biscuits, which showed a range of 144–781 μg/kg (Verma and Yadav, 2022). According to EFSA, the average acrylamide content of biscuits is estimated to be 201 μg/kg, with the 95th percentile reaching 810 μg/kg. It is important to note that acrylamide content can vary significantly between different brands and type of biscuits (EFSA, 2015a). All other cereal-based foods consumed during breakfast had lower acrylamide levels and all of them were below BL described in the European Regulation (EC, 2017) (Table 2). The results for breakfast cereals are among the lowest values reported for commercial breakfast cereals marketed in Spain (from <20 to 639 μg/kg) (Mesías et al., 2019a), and they are lower than the range described for samples from the Slovenian market (220.2–498.5 μg/kg) (Mencin et al., 2020). Even the pastries (bagel and muffins) examined

Table 2
Acrylamide content (μg/kg), edible serving (g) and acrylamide contribution (μg/serving) to both lower and upper bound scenarios in meals compounding breakfast and lunch of menus provided by school canteens.

Meal	n*	FoodEx2 code	Acrylamide content (μg/kg)		Edible serving (g)	Acrylamide contribution (μg/serving)	
			mean	SD		mean	SD
School 1							
Breakfast							
Biscuits	4	A009V	646	44	42	27.1	0.6
Cornflakes	4	A00DD	31	1	37	1.1	0.1
Chocolate puffed rice breakfast cereals	2	A00DR#F04.A034J	71	5	31	2.2	0.2
Lunch							
Beans with sausage stew	1	A03VT	16		523	8.4	
Pilau rice	1	A040M	22		203	4.5	
Sauteed peas with egg and ham	1	A012G#F04.A00GZ$F04.A022T$F04.A031G$F28.A07GT	20		318	6.4	
Noodles with red pepper and garlic	1	A040M#F04.A00JA$F04.A00GZ	16		335	5.4	
Lentils stew	1	A00QD#F28.A07GM	19		564	10.7	
Bread	10	A0DRD	33	24	25	0.8	0.8
School 2							
Breakfast							
Biscuits	2	A009V	680	2	28	19.0	0.1
Whole wheat biscuits	2	A00AA	117	1	45	5.3	0.1
Puff pastry biscuits	2	A00CC	111	2	22	2.4	0.1
Lunch							
Potato and pork ribs stew†	1	A03VJ	16		276	4.4	
Breaded burger	1	A022K#F04.A03XF$F04.A07HK$F28.A07GS	17		76	1.3	
French fries	1	A0BYV	41		85	3.5	
White beans with sausage stew	1	A03VT	25		264	6.6	
Tuna omelette	1	A03YN#$F04.A03YN	43		79	3.4	

Only meals with an acrylamide concentration higher than LOQ (15 μg/kg) are detailed. *n refers to the times that food is consumed during the two weeks of trial. †All the food servings were edible except for potato and pork ribs stew where the non-edible portion was removed (total serving: 282 g). Data are mean ± SD of at least two analytical duplicates.

showed non-quantifiable levels of the contaminant, as did fruits, juices, milk and soluble cocoa.

The lunches served at participating school showed generally low acrylamide concentrations (foods/meals with non-quantifiable acrylamide levels were not considered), ranging from 16 μg/kg (found in bean and sausage stew, noodles with red pepper and garlic, and potato and pork rib stew) to 43 μg/kg (detected in tuna omelette). Only the bread from school 1 had quantifiable levels of the contaminant, with a mean value of 33 μg/kg, which was lower than the BL for wheat-based bread (50 μg/kg) (EC, 2017). The acrylamide content in bread can vary based on factors such as bread type, ingredients used and baking conditions. According to EFSA, the average acrylamide content of bread is estimated to be 42 μg/kg, with the 95th percentile at 156 μg/kg (EFSA, 2015a). Even though French fries are known to be one of the major sources of exposure to acrylamide in the diet (El Tawila et al., 2020), the French fries analysed in the schools of this study did not exhibit high levels of acrylamide. Acrylamide was not detected in French fries served at school 1, and very low concentrations were found in those served at school 2 (41 μg/kg), which were significantly lower than the BL for this foodstuff (500 μg/kg) (EC, 2017). It is necessary to point out that French fries at school 1 were prepared from frozen par-fried potatoes, which usually undergo blanching and light frying treatments before freezing, reducing the precursors and the subsequent acrylamide formation during frying (Negoiță et al., 2022). With the exception of breaded hamburgers and tuna omelette, all other foods found to contain acrylamide were stews or stir-fried dishes. The acrylamide content in these meals may be related to the presence of fried or sautéed vegetables, such as onions or peppers, which are known to contain sugars and asparagine that promote the acrylamide formation during heat treatments (Başaran, 2022). Additionally, spices or seasonings added to these meals, like red paprika, turmeric or black pepper, may also contribute to acrylamide content since they have been reported to contain significant amounts of the contaminant (Jeong et al., 2020; Wongthanyakram et al., 2020). Previous studies have established similar findings (Delgado-Andrade et al., 2012), identifying acrylamide levels of 5 μg/kg in vegetable soup and 20 μg/kg in bean and rice stew (data expressed in dry matter). In breaded hamburgers, acrylamide presence is linked to the bread used in the product coating (Guerra-Hernández, 2016). For tuna omelette, the contribution of other ingredients included in the meal, such as fried onion, cannot be ruled out as potential sources of acrylamide.

When considering only the edible serving of each food/meal, biscuits were found to have the highest acrylamide levels (19.0–27.1 μg/serving) (Table 2), followed by stew-based meals, with concentrations ranging between 6.6 and 10.7 μg/serving. A previous study conducted by our research team described a lower figure for bean stew prepared with rice (2.4 μg/serving) (Delgado-Andrade et al., 2012). All other meals contributed to a lesser extent to the daily intake of the contaminant. Regarding bread, only the bread included in the menu served at school 1 contributed to daily acrylamide intake, with an estimated 0.8 μg/serving. This value is significantly lower than the 5.2 μg/serving reported in a previous investigation (Delgado-Andrade et al., 2012).

3.3. Daily acrylamide intake from school canteen menus and the contribution of individual foods/meals. LB and UB scenarios

Based on the acrylamide levels calculated for each food or meal, the daily acrylamide intake from breakfast and lunches served in school menus over the course of the two-week trial was estimated. At this point it is important to mention that, as the contribution in the complete edible portion was considered for this estimation, the results would represent the maximum daily acrylamide intake from each school canteen. Two scenarios were considered: the LB scenario, using only data from Table 2 and the UB scenario, which incorporated data from both Tables 2 and 3. In each scenario, average values were calculated for a one-week period and for the ten-day period of data collection, in accordance with daily contaminant intake (Table 4). In the LB scenario, the daily acrylamide intake ranged from 2.0 to 33.3 μg/day at school 1 and from negligible to 19.1 μg/day at school 2. The weekly averages for

Table 3
Edible serving (g) and acrylamide contribution (μg/serving) to the upper bound scenario in processed meals with acrylamide levels below LOQ compounding breakfast and lunch of menus provided by school canteens.

Meal	n*	FoodEx2 code	Edible serving (g)	Acrylamide contribution (μg/serving)
School 1				
Lunch				
Spaghetti bolognese	1	A040Q	368	5.5
Battered squid rings	1	A02JJ#F28.A07HL$F28.A07GV	221	3.3
Beef burger	1	A03XF	150	2.3
'A banda' rice	1	A041D	328	4.9
French omelette	2	A03YN	109	1.6
Roasted chicken drumsticks	1	A022C#F28.A07GY	126	1.9
French fries	1	A0BYV	100	1.5
Lean pork and potato stew	1	A03VJ	430	6.4
Pomfret in tomato sauce	1	A02AP#F04.A044C	251	3.8
Breaded monkfish	1	A02BJ#F28.A07HK$F28.A07GS	229	3.4
Zucchini cream	1	A03XY	495	7.4
Grilled pork loin with sauteed vegetables	1	A06GH#F04.A03YB	196	2.9
Tuna in tomato sauce	1	A02DX#F04.A044C	199	3.0
Sauteed pasta	1	A040R	183	2.7
Mixed paella	1	A041D	322	4.8
Traditional chickpea-based stew prepared with meat and vegetables	1	A03VK#F04.A00SL$F28.A07GM	337	5.1
School 2				
Breakfast				
Soluble cocoa	10	A03HG	4	0.1
Bagel	1	A00BR	33	0.5
Muffin	1	A00BC	32	0.5
Lunch				
Bread	10	A0DRD	45	0.7
Pasta with sausage, cheese and tomato sauce	1	A040Q#F04.A025C$F04.A06BN	452	6.8
Baked cod	1	A02BV#F28.A07GX	88	1.3
Sauteed chard with ham	1	A03XX#F04.A00MX$F04.A022T$F28.A07GT	128	1.9
Turkey stew	1	A03VY#F04.A023T	122	1.8
White rice	1	A040M	166	2.5
Breaded horse mackerel fillets†	1	A02CN#F28.A07HK	82	1.2
Carrot cream	1	A03XY	238	3.6
Battered chicken cutlets	1	A01SP#F28.A07HL	123	1.8
Traditional chickpea-based stew prepared with meat and vegetables	1	A03VK#F04.A00SL$F28.A07GM	326	4.9
Sauteed peas with bacon	1	A03XX#F04.A012H$F04.A022X$F28.A07GT	232	3.5
Baked pomfret in tomato sauce	1	A02AP#F04.A044C$F28.A07GX	211	3.2
Pasta salad	1	A042H	145	2.2
Brothy rice with chicken and vegetables	1	A041J#F04.A01SP	215	3.2
Breaded hake	1	A02CB# F28.A07HK	46	0.7
Sauteed of boiled chard and potato	1	A03XX#F04.A00MX$F04.A00ZT$F28.A07GL $F28.A07GT	119	1.8
Roasted chicken drumsticks†	1	A022C#F28.A07GY	140	2.1

Acrylamide concentration in all the samples were between LOD and LOQ, consequently a value of 15 μg/kg was assigned for all of them. *n refers to the times that food is consumed during the two weeks of trial. †All the food servings were edible except for roasted chicken drumsticks and breaded horse mackerel fillets where the non-edible portion was removed (total serving: 209 g and 112 g, respectively).

school 1 were 15.7–16.4 μg/day, while for school 2, they were 6.2–8.3 μg/day. Despite these differences, the mean acrylamide intake was not found to significantly differ between schools (16.1 *vs*. 7.3 μg/day at schools 1 and 2, respectively; $p = 0.072$). When considering the students from both schools together, the accumulated mean acrylamide intake was 11.7 μg/day. In the UB scenario, the daily intake was notably higher compared to the LB scenario. At school 1, the daily intake ranged from 9.3 to 38.1 μg/day, while at school 2, it ranged from 5.1 to 28.0 μg/day. The mean acrylamide intakes for both weeks were substantially different but without statistical difference (22.4 *vs*. 12.5 μg/day for school 1 and 2, respectively). The accumulated mean acrylamide intake was 17.4 μg/day for both centres, which was not significantly different from the figure obtained in the LB scenario. The highest weekly acrylamide intake levels occurred on days when biscuits were consumed at breakfast (Table 2), being the only source of acrylamide exposure at school 2 in the LB scenario. Although to a lesser extent, notable acrylamide exposures were observed on days when stews were included on the menu (Table 4). The acrylamide content of these meals was relatively low (16–25 μg/kg), but the portion sizes were larger than for other foods/meals, resulting in a higher total content per serving (6.6–10.7 μg/serving) (Table 2). On all remaining days, acrylamide intake levels were lower, with negligible exposure to the contaminant recorded on 3 days in the case of school 2 in the LB scenario.

The contribution of foods and meals included in breakfast and lunch to daily acrylamide intake considering both the LB and UB scenarios is presented in Fig. 2. In the LB scenario, breakfast accounted for 73.3% of daily acrylamide intake, primarily due to biscuit consumption. Cereal-based foods, such as biscuits and breakfast cereals, were the main contributors to breakfast intake in this scenario. On the other hand, lunches were responsible for 26.7% of daily acrylamide intake. Among lunch items, the main contributors were the main course, especially stews, followed by side dishes like French fries or pilau rice; second courses and, finally, bread. However, in the UB scenario, the situation changed significantly. Now, the major contribution came from lunches, accounting for 65.4% of daily acrylamide intake, while breakfast provided the lowest contribution at 34.6%. This shift highlights the significance of including foods and meals with minimal levels of acrylamide, which were assigned the LOQ value, in a comprehensive assessment of dietary

Table 4
Acrylamide intake through the consumption of breakfasts and lunches provided by the school canteens.

	Acrylamide intake (μg/day). LB scenario						
	Monday	Tuesday	Wednesday	Thursday	Friday	Mean	SD
School 1							
Week 1	27.9	14.7	3.0	8.3	27.9	16.4	11.3
Week 2	33.3	2.0	13.5	2.0	27.9	15.7	14.5
2 weeks mean						**16.1a**	**12.3**
School 2							
Week 1	19.1	nd	6.8	nd	5.3	6.2	7.8
Week 2	19.1	4.8	2.4	nd	15.4	8.3	8.4
2 weeks mean						**7.3a**	**7.7**
Both schools						**11.7**	**10.9**
	Acrylamide intake (μg/day). UB scenario						
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Mean**	**SD**
School 1							
Week 1	36.7	17.0	9.6	11.7	38.1	22.6	13.8
Week 2	36.8	12.4	19.2	9.3	32.9	22.1	12.2
2 weeks mean						**22.4a**	**12.3**
School 2							
Week 1	28.0	7.5	8.8	6.6	10.9	12.3	8.9
Week 2	26.5	8.2	7.1	5.1	16.1	12.6	8.8
2 weeks mean						**12.5a**	**8.4**
Both schools						**17.4**	**11.4**

LB: lower bound. UB: upper bound. nd: non-detected. Same letters in the values for 2-week mean indicate no significant differences between schools for LB scenario ($p = 0.072$) and UB scenario ($p = 0.052$).

acrylamide intake. The impact of this contribution could be substantial, to the extent that the combined intake of these highly consumed food items might outweigh that of those considered the main sources of acrylamide, which are consumed less frequently.

The findings reported by Branciari et al. (2020) and Hilbig et al. (2004) are consistent with the results obtained in the LB scenario of the present research. In the study by Branciari et al. (2020), acrylamide exposure was estimated in Italian schoolchildren consuming a canteen menu. In this case, the daily acrylamide contribution of all cereal products (pasta, breakfast cereals, bread and bakery products) ranged from 30.2 to 32.7%, depending on the age of schoolchildren. In that study, higher intake was associated with meals served at lunch (62.9–73.2%), with approximately half of all acrylamide content being provided by potato-based products (30.2–34.8%). Similarly, in the investigation by Hilbig et al. (2004), which recorded the whole diet of children and adolescents, the main contributors to acrylamide intake were bread (18–46%), pastries (16–35%) and potato products (7–35%).

3.4. *Dietary acrylamide exposure at school canteens*

The assessment of mean dietary exposure to acrylamide in students attending school canteens was conducted based on three age ranges: 3–5 years, 6–9 years and 10–12 years (Table 5). In consideration of the influence of body weight and taking into account that the same portions of foods consumed were applied for the three age groups, acrylamide exposure was found to be higher in the youngest students and lower in early adolescents. Although more than 50% differences emerged in exposure between these age groups, no statistically significant differences were found between groups in the LB scenario. In contrast, in the UB scenario, significant differences in exposure were observed in school 1 ($p = 0.033$) and when considering data from both schools together ($p = 0.007$). As expected, due to the acrylamide contribution from each

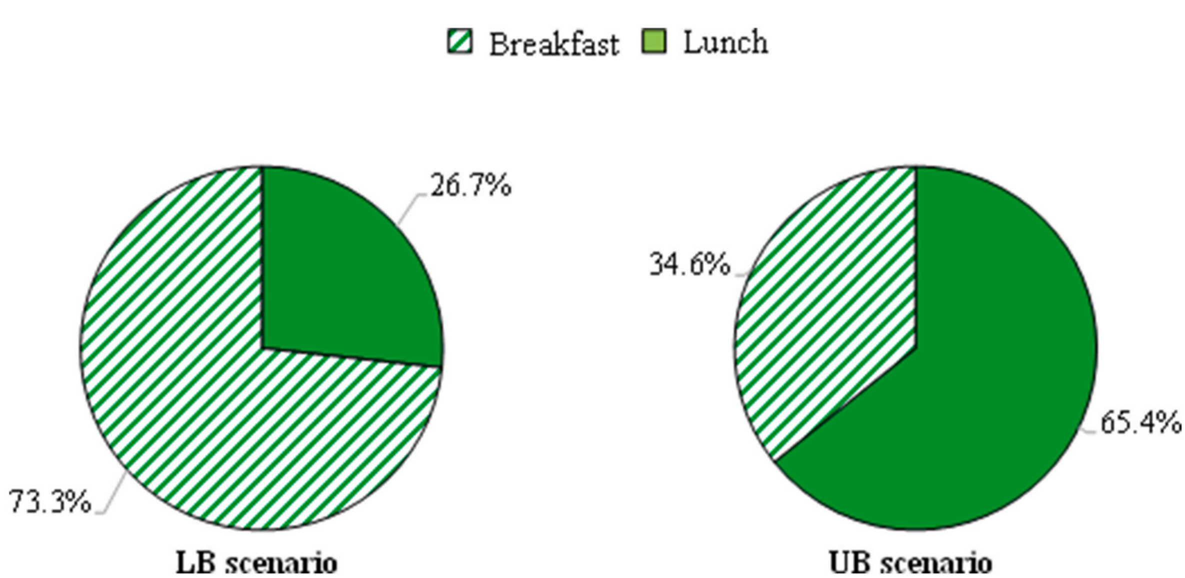

Fig. 2. Contribution of the foods and meals included in breakfast and lunch to the daily acrylamide intake (%). LB: lower bound. UB: upper bound.

Table 5
Dietary exposure to acrylamide at the school canteens and associated risk assessment.

	Schoolchildren age ranges													
	3-5 y				6-9 y				10-12 y				P value*	
	LB		UB		LB		UB		LB		UB		LB	UB
School 1	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**		
Exposure (μg/kg bw per day)	0.77	0.66	1.18b	0.65	0.54	0.46	0.86Bab	0.47	0.36	0.31	0.56a	0.31	0.146	0.033
MOE for neurological effects	560		365		796		500		1179		769			
MOE for neoplastic effects	221		144		315		198		466		304			
School 2														
Exposure (μg/kg bw per day)	0.35	0.40	0.66	0.44	0.25	0.28	0.48A	0.32	0.17	0.19	0.31	0.21	0.357	0.092
MOE for neurological effects	1233		655		1752		897		2595		1379			
MOE for neoplastic effects	487		259		692		354		1026		545			
P value†	0.072		0.051		0.073		0.049		0.069		0.051			
Both schools														
Exposure (μg/kg bw per day)	0.59	0.58	0.92b	0.60	0.43	0.41	0.67 ab	0.44	0.28	0.27	0.44a	0.28	0.072	0.007
MOE for neurological effects	735		469		995		641		1547		989			
MOE for neoplastic effects	291		186		393		253		612		391			

LB: lower bound. UB: Upper bound. Different lowercase letters mean significant differences between the acrylamide exposure of the three age ranges considered in each school for each scenario ($p < 0.05$)*. Different capital letters mean significant differences in the acrylamide exposure for each age range between the two schools ($p < 0.05$)†.

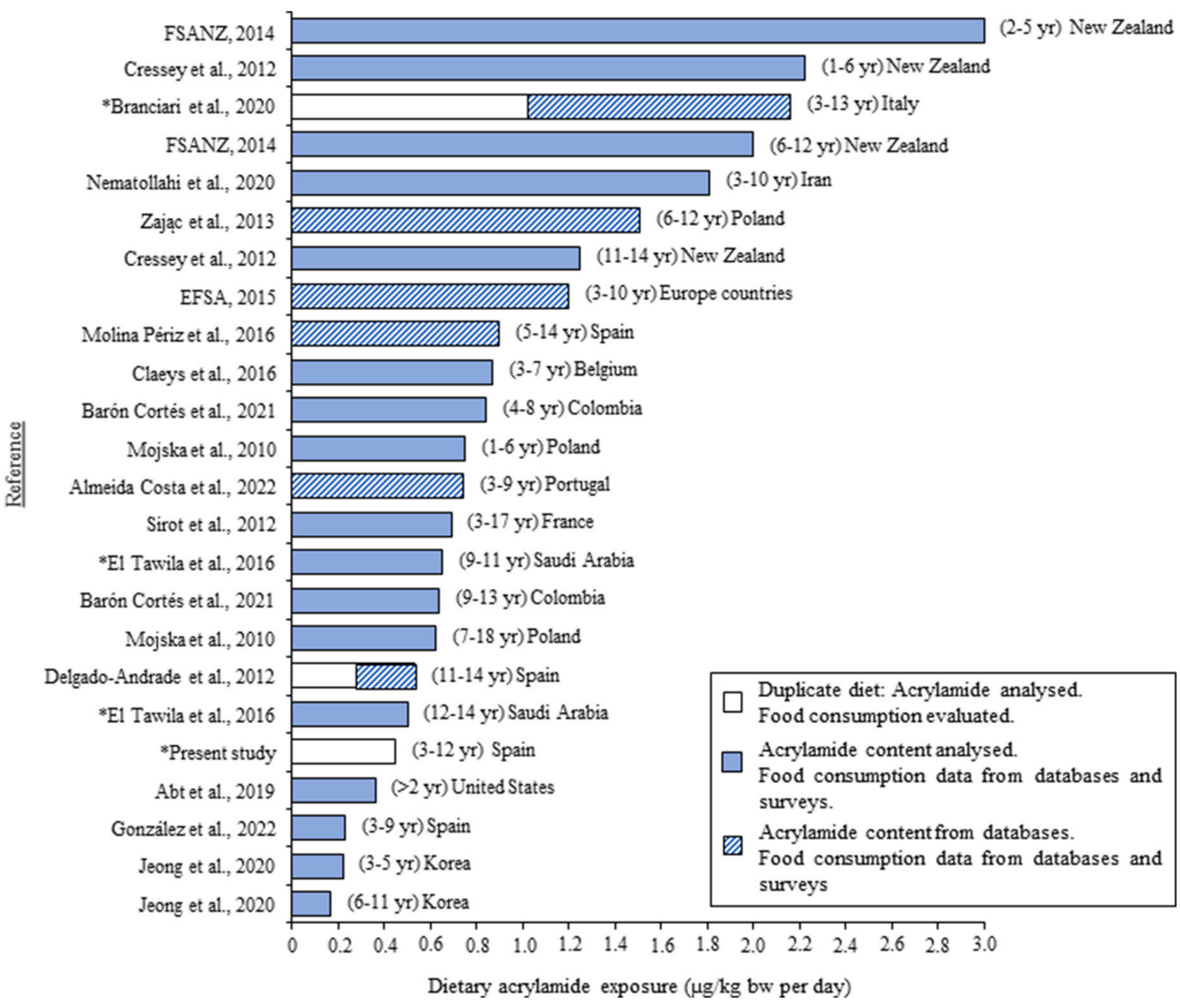

Fig. 3. Dietary acrylamide exposure reported by different studies from different countries. *These studies do not describe the acrylamide exposure from the total diet, but only from part of the daily diet (Abt et al., 2019; Almeida Costa et al., 2022; Barón Cortés et al., 2021; Branciari et al., 2020; Claeys et al., 2016; Cressey et al., 2012; Delgado-Andrade et al., 2012; EFSA, 2015, FSANZ, 2014; González et al., 2022; Jeong et al., 2020; Mojska et al., 2010; Molina Périz et al., 2016; Nematollahi et al., 2020; Sirot et al., 2012; Zając et al., 2013).

school canteen, exposure at school 2 (range: 0.17–0.35 μg/kg bw per day for LB and 0.31–0.66 μg/kg bw per day for UB) was lower than at school 1 (range: 0.36–0.77 μg/kg bw per day for LB and 0.56–1.18 μg/kg bw per day for UB), although significant differences only emerged when comparing the 6–9 y age groups in the UB estimation. Considering overall data collected from both schools, mean acrylamide exposures were 0.28 μg/kg bw per day (10–12 y), 0.43 μg/kg bw per day (6–9 y) and 0.59 μg/kg bw per day (3–5 y) in the LB scenario and 0.44 μg/kg bw per day (10–12 y), 0.67 μg/kg bw per day (6–9 y) and 0.92 μg/kg bw per day (3–5 y) in the UB scenario. The finding that higher exposure was associated with lower body weight reaffirms the need to control the consumption of processed foods among pre-schoolers (3–5 y). This is because higher acrylamide exposure in this age group may increase the risk associated with acrylamide exposure in younger school children by 25% (when compared with students aged 6–9 y) and 50% (when compared with students aged 10–12 y).

Any comparison between data reported in the present study and findings from existing literature should be made with caution due to the different methods employed for estimating dietary acrylamide exposure. On the one hand, food consumption data are usually collected from surveys, which fail to represent real intake within a population and, to an even lesser extent, within specific groups. The most frequently used surveys are: a) 24-h dietary recall, which provides individual data on food consumption and culinary techniques as remembered and perceived by respondents; b) food record diaries, where participants record information about the portions of food consumed but are hampered since they require information to be gathered over a number of days, and; c) food frequency questionnaires, which provide more global information about the frequency of consumption of specific foods in the diet and lack precision and may not capture the specific cooking techniques used (Sirot et al., 2012). On the other hand, some assessments of acrylamide content rely on data collected from existing literature rather than direct food measurements. This approach presents challenges in assigning single acrylamide values to individual foods due to the influence of various factors, such as the content of precursors in the raw material, the degree of processing, culinary technique applied, etc. Our research team has reported large variability in estimated acrylamide levels for the same food matrix especially across different production settings, including the food industry (Mesías et al., 2019b, 2020b; 2022), households (Mesías et al., 2018), restaurants (Mesías et al., 2019c) or collective catering (Mesías et al., 2020a). This variation underscores the impact of food preparation settings on dietary exposure to the contaminant (González-Mulero et al., 2021). To obtain accurate information on both real food consumption and acrylamide content in foods, the most suitable method is the duplicate diet approach. This method involves collecting duplicate samples of the foods consumed by an individual or a group over a specific period. The collected samples are then analysed for their acrylamide content using appropriate analytical techniques, providing reliable data on actual food consumption and acrylamide exposure (Shim et al., 2014; Porca Fernández et al., 2016).

Levels of acrylamide exposure reported in existing literature for similar age ranges as the mean value evaluated in the present investigation are presented in Fig. 3. It is observed that only two studies applied a duplicate diet methodology (Delgado-Andrade et al., 2012; Branciari et al., 2020). Most prior research reports acrylamide exposure in relation to the overall diet, with only a few studies evaluating specific aspects of daily consumption. This was the case in the present investigation, where only foods and meals served at breakfast and lunch were considered. Therefore, the present estimations (considering only LB in this comparison) are likely to be lower than those described in most other studies, specifically, rising from 0.5 μg/kg bw per day in the present study to 3 μg/kg bw per day in other studies. However, despite not considering the overall diet, the present findings revealed higher values than those reported in some other works, which recorded estimates ranging from 0.17 to 0.36 μg/kg bw per day (Abt et al., 2019; Jeong et al., 2020; González et al., 2022). When comparing with the estimations calculated by Branciari et al. (2020) for acrylamide exposure through breakfast and lunch menus served at school canteens, the present findings in Spanish schoolchildren indicate a lower level of exposure to this contaminant. Our estimations were also lower than those reported for Arab school students through the evaluation of foods served at school cafeterias. The differences in this case may be attributed to the fact that, while foods were analysed to determine acrylamide content, food consumption was estimated using food frequency questionnaires (El Tawila et al., 2017). This method may lead to overestimation of outcomes.

3.5. Risk assessment

As a means of evaluating the risk associated with acrylamide exposure through school canteen menus, MOE values were calculated according to age and school centre. MOE values provide an indication of the level of concern posed by the presence of a potentially harmful compound in food and help in keeping exposure as low as possible. The EFSA Scientific Committee states that MOE values lower than 10,000 for genotoxic and carcinogenic substances are highly concerning from a public health perspective. Similarly, MOE values lower than 125 indicate risk of non-neoplastic effects (i.e. neurological effects) (EFSA, 2015a).

MOE values calculated for neurological and neoplastic effects in student at different ages are presented in Table 5. Concerning neurological effects, all MOE values were above the reference value of 125 (range: 560–2595 in LB scenario, 365–1379 in UB scenario), indicating no risk. However, with regards to neoplastic effects, all values were well below the reference value of 10,000 (range: 221–1026 in LB scenario, 144–545 in UB scenario), indicating a high level of concern. It is important to note that these estimations only considered acrylamide exposure from breakfast and lunch consumption and, therefore, underestimated the total exposure to the contaminant. As expected, lower MOE values have been reported in European children for both neurotoxic (269–475) and neoplastic effects (106–189) when the total diet was considered (EFSA, 2015a). However, data of similar order of magnitude as reported in the present research have been documented in children in other studies, even when estimating total daily diet (Barón Cortés et al., 2021; Almeida Costa et al., 2022). In summary, due to the low body weight of the participating children, even the very small acrylamide exposure found in relation to just two of the meals consumed in a day, resulted in some concern outcomes regarding the neoplastic effects of the contaminant. This concern would have been even greater if the overall daily diet had been considered.

4. Conclusions

The present research assessed dietary acrylamide exposure through menus (breakfast and lunch) provided by school canteens in Spanish children using the duplicate diet method, following the latest EFSA recommendations. The investigation acknowledges certain considerations in its design, such as using the same standard portion of foods for all age ranges addressed and not accounting left-overs, which may overestimate the exposure particularly for the youngest children, and relying on literature data for body weights of different populations. Acrylamide was detected in only 21% of analysed foods/meals. Cereal-based products, especially biscuits, were the greatest contributors to acrylamide intake, followed by stews (due to their large portion size), fried potatoes and seasonings. Considering the LB scenario, breakfasts were the major contributors to daily acrylamide intake, while lunches were the main responsible in the UB scenario. The acrylamide intake through school canteen menus, along with the small body weight of students, resulted in low MOE values, indicating a risk of neoplastic effects in both the LB and the UB scenarios. This concern would have been even greater if the overall daily diet had been considered. The main outcome highlighted that every type of diet, even those that are well-balanced, diverse, and composed of low-acrylamide foods, still leads to some level of exposure to this contaminant. Therefore, this finding emphasises the importance of incorporating this additive exposure into the assessment of acrylamide exposure risks.

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CRediT authorship contribution statement

Lucía González-Mulero: Data curation, Formal analysis, Validation, Writing – original draft. **Cristina Delgado-Andrade:** Methodology, Conceptualization, Funding acquisition, Investigation, Validation, Writing – review & editing. **Francisco J. Morales:** Methodology, Funding acquisition, Investigation, Writing – review & editing. **Marta Mesías:** Methodology, Conceptualization, Funding acquisition, Investigation, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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