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Risk assessment of the exposure of Spanish children to acrylamide using human biomonitoring☆

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ABSTRACT

Acrylamide (AA) is an organic contaminant that naturally forms in starchy foods during high-temperature cooking under low-moisture conditions. It is mainly produced from the sugars and amino acids present in food by the Maillard reaction. When humans are exposed to AA, AA is eliminated in the urine as mercapturic acid conjugates, primarily including N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA), N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA3), and N-acetyl-3-[(3-amino-3-oxopropyl)sulfinyl]-L-alanine (AAMA-Sul), which are used as exposure biomarkers of AA in human biomonitoring studies. Although the carcinogenic effects of AA on humans have not been demonstrated yet, some studies have shown that AA may negatively affect children's health.

The main objective of this study was to evaluate the exposure of Spanish children (n = 612) to AA. For this purpose, the levels of AAMA, AAMA-Sul, and GAMA3 in first-morning urine samples were analyzed by "dilute and shoot" and liquid chromatography coupled to tandem mass spectrometry. The three metabolites were detected in all the children involved in this study in the following order (geometric mean (GM)): AAMA (79 ng ml^{-1}) > AAMA-Sul (28 ng ml^{-1}) > GAMA3 (18 ng ml^{-1}). Statistical analysis suggested that the intake of fried potato products and biscuits could be associated with higher levels of AA metabolites in urine. Estimated daily intakes of AA in the children under study were in the range of 1.2–1.5 μg AA·kg-body $weight^{-1}$·day^{-1} (GM). Risk assessment calculations indicate that the health risk of AA exposure cannot be overlooked and the exposure of Spanish children to AA should be closely monitored.

1. Introduction

Acrylamide (AA) is a volatile contaminant with various industrial applications and has been classified as a "probable human carcinogen" (Group 2A) by International Agency for Research on Cancer (IARC) (HBM4EU, 2019a; IARC, 1994).

In 2002, the discovery of the formation of AA in carbohydrate-rich foods at temperatures above 120 °C and under low-moisture conditions (for example, during frying, roasting, and baking) via the Maillard reaction had attracted significant attention of the scientific society and public health authorities (WHO, 2002). In fact, the general population is exposed to AA mainly through the consumption of potato crisps, French fries, bakery products (bread and biscuits), and coffee (EFSA, 2015) because of their composition, processing conditions, or high consumption frequency (Rifai and Saleh, 2020).

Although there are some non-dietary sources, such as tobacco smoke, diet is speculated to be the main source of AA exposure in the non-smoking population (EFSA, 2015; Rifai and Saleh, 2020). Consequently, national food authorities in the European Union countries are continuously monitoring the presence of AA in certain food commodities following the Recommendation 2007/331 of the European Commission (European Commission, 2007). In 2015, based on the abovementioned

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data, the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) published a scientific opinion assessing the risk associated with the exposure of European citizens to AA (EFSA, 2015). However, this approach has two primary disadvantages: i) it only considers dietary intake and does not include other possible routes or sources of AA exposure and ii) the presence of AA in food may not reflect the actual uptake of AA by the human body, since it depends not only on individual eating habits in terms of cooking methods, portion size and frequency of consumption (Timmermann et al., 2021), but also on the protein content of each individual diet (Schabacker et al., 2004). A complementary and more suitable alternative for evaluating human exposure to AA and the risk associated with it could be human biomonitoring (HBM). This approach facilitates an accurate calculation of the internal dose of AA and considers all pathways (namely, inhalation, ingestion, and dermal contact) of AA exposure by measuring the levels of AA metabolites in biological matrices such as urine and blood (Louro et al., 2019).

After the absorption of AA by the human body, AA is distributed to all tissues and rapidly metabolized via one of the following two pathways: i) it may react with glutathione and produce the mercapturic acid *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine (AAMA) or ii) it may be oxidized to glycidamide (GA) by cytochrome P450 (Kocadağli and Gökmen, 2016; Rifai and Saleh, 2020). GA can be further hydrolyzed to glyceramide (2,3-dihydroxypropionamide) by an epoxide hydrolase (Albiach-Delgado et al., 2022; Hartmann et al., 2011), and can also conjugate with glutathione to form *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA3), which are then excreted in the urine (Kocadağli and Gökmen, 2016; Rifai and Saleh, 2020). Additionally, AAMA can be oxidized to its corresponding sulfoxide, generating acetyl-3-[(3-amino-3-oxopropyl)sulfinyl]-L-alanine (AAMA-Sul).

The three mercapturic acids are the major urinary metabolites of AA, which have been proposed as suitable biomarkers of short-term exposure to AA in HBM studies (Hays and Aylward, 2008; Kocadağli and Gökmen, 2016).

Generally, very few HBM studies have been reported on internal AA exposure in humans, specifically, those belonging to vulnerable groups, including infants and young children, which are of special concern as they need more food, water, and air per kilogram of body weight (kg-bw) as compared to the cases of adults, resulting in a higher exposure of these groups to AA (Hartmann et al., 2008; Mielech et al., 2021). Although there is no evidence of neoplastic effects of AA on humans (EFSA, 2015), two studies conducted in several European populations have shown that AA intake may negatively affect the development of children (Duarte-Salles et al., 2013; Pederson et al., 2012). Other health conditions, such as cardiovascular diseases (Hsu et al., 2020) and oxidative stress damage (Kuang et al., 2021; Qian et al., 2021), have also been associated with the exposure of this population group to AA.

To date, among the European countries, only Germany has reported quantifiable data on the urinary levels of AAMA and GAMA3 in children and adolescents (Hartmann et al., 2008; Heudorf et al., 2009; Schwedler et al., 2021). Indeed, HBM for Europe (HBM4EU) initiative included AA in its 2nd list of priority substances (2018) because of not only the existing gap in its potential adverse effects on humans, but also the lack of data on internal AA exposure in the European population (HBM4EU, 2018). Therefore, considering the potential carcinogenic activity of AA and the scarcity of the HBM studies focused on the exposure of children to AA, obtaining data on the internal exposure of this vulnerable group to AA is of substantial interest.

Herein, data on the internal exposure of Spanish children to AA are presented and the corresponding risk is assessed for the first time. Moreover, potential exposure factors were identified by regression analysis.

2. Materials and methods

2.1. Study design: recruitment, data, and sample collection

This study was performed in the framework of BIOVAL program, a HBM cross-sectional study promoted by the Health Department of the Regional Government of Valencia (Spain) in 2016. The main objective of this program was to more comprehensively characterize the exposure profiles of children (5–12 years old) from the Valencian Region (Spain) to food contaminants. A complete description of the different fieldwork stages (that is, logistics, recruitment, sampling, and data collection) of the BIOVAL program can be found in the literature (Pérez et al., 2017).

Briefly, a total of 666 children from different primary schools throughout the three provinces (Castelló, València, and Alacant) of the Valencian Region participated in the BIOVAL study. The selection requirements were as follows: i) age between 5 and 12 years; ii) no diseases related to the urinary system; and iii) home residence located in the Valencian Region. Before sampling and data collection, informed consent was obtained from the parents/guardians of each volunteer.

The families were provided with all the necessary materials and detailed instructions for sample collection. First-morning urine samples were acquired by all volunteers in 100-ml polypropylene containers using disposable gloves. All urine samples were transported by the BIOVAL staff and refrigerated (4 °C) until storage in 10-ml aliquots at −80 °C in the Biobank for Biomedical Research and Public Health of the Valencian Community (IBSP-CV Biobank, Spain; PT13/0010/0064). Additionally, sociodemographic information and food consumption habits (monthly consumption frequency and 72-h recall) of the children were obtained via a self-administered questionnaire.

The BIOVAL program was approved by the Ethics Committee for Clinical Research of the Directorate General of Public Health and the Centre for Advanced Research in Public Health of the Valencian Government. The participants were notified that the information would be published only for scientific purposes and would not include any personal details. The collection, handling, transfer, and analysis of personal data were conducted by secure settings. Moreover, these data were destroyed after final processing, which was carried out in compliance with ethics and legal aspects at the national and European level, particularly the General Data Protection Regulation.

2.2. Chemical analysis

Methods for the preparation and analysis of samples and details of the standards, reagents, analysis conditions, and quality assurance/quality control criteria used to examine the levels of AAMA, AAMA-Sul, and GAMA3 in the urine samples have been comprehensively described in the literature (Fernández et al., 2022).

Briefly, the homogenized sample (200 μl) was diluted with the internal standard solution and water (0.01% acetic acid) and then injected into a Vanquish high-performance liquid chromatography (Thermo Fisher Scientific Inc.; San Jose, CA, USA) equipped with an Atlantis dC_{18} column (150 mm × 2.1 mm, 2.6 μm, 100 Å) (Waters, Milford, MA, USA) and coupled to an Altis™ triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc.; San Jose, CA, USA); the spectrometer was operated using an electrospray ionization source in the negative-ion mode. All metabolites presented a limit of quantification (LOQ) of 0.25 ng ml^{-1}. Chromatograms of different concentration levels (LOQ, 50 and 1000 ppb) in the calibration curve and of a real urine sample, showing the chromatographic separation of AAMA-Sul, GAMA3 and AAMA, are presented in Fig. S1. During the analysis of the BIOVAL urine samples, the external quality assurance of the results was evaluated through participation in the 66th German External Quality Assessment Scheme (G-EQUAS). As a result, |Z| (absolute Z-scores) between 0.15 and 1.35 for AAMA, and between 0.32 and 0.59 for GAMA3 were obtained, which were classified as satisfactory results ($|Z| \leq 2$). Quantification of AAMA-Sul was not assessed since it was not included in the G-EQUAS 66.

Creatinine (Cre) analysis was performed using the Jaffé's reaction at the University Hospital Doctor Peset (València, Spain) for two purposes: i) to correct urine dilution differences among samples and ii) to exclude those urine samples with abnormal Cre concentrations. As a 'normal' urinary Cre range for children has not been described yet in the literature, the 'normal' values for adults (30–300 mg dl^{-1}) were considered (Barr et al., 2005).

2.3. Statistical analysis

Initially, the urinary levels of AA metabolites adjusted by volume ($ng{\cdot}ml^{-1}$) and Cre ($\mu g{\cdot}g^{-1}$ Cre) were summarized using the following descriptive statistics: detection frequency (DF, percentage of samples above LOQ), geometric mean (GM), 25th, 50th, 75th, and 95th percentiles (P25, P50, P75, and P95, respectively), minimum and maximum values, and standard deviation (SD).

Sociodemographic characteristics of the participants and dietary habits related to AA exposure (namely, exposure variables) were extracted from the questionnaires and described by absolute frequencies and percentages for qualitative variables (with two or more categories) and median, minimum, and maximum values for the quantitative variables.

SPSS statistics (version 26.0, 2019) was used to conduct statistical analysis of the data. A Spearman correlation matrix was employed to investigate the correlations among AAMA, AAMA-Sul, and GAMA3. Urinary concentrations of these AA biomarkers were converted to $nmol{\cdot}l^{-1}$ and added together to obtain the sum of urinary AA metabolite levels ($\sum$UAAM). Due to the non-normal distribution of the data, the logarithm of $\sum$UAAM was used as a dependent variable in the construction of a regression model based on the corrected Akaike information criterion (Konishi and Kitagawa, 2008) to explore associations between $\sum$UAAM and the exposure variables, including Cre as an independent variable (HBM4EU, 2019b). Qualitative variables having categories with frequencies higher than 90% were not included in the exposure variables. A p-value of 0.05 was set as a cutoff for significance. In the final model, the regression coefficients and their 95% confidence intervals for each significant variable reflected the change in the logarithm of $\sum$UAAM of the target population.

To check the assumptions of normality, non-autocorrelation, homoscedasticity, and non-multicollinearity, the Shapiro–Wilk test, the Durbin–Watson statistic, scatter plots, and tolerance and condition indices, respectively, were employed.

2.4. Calculation of estimated daily intake (EDI) of AA

Daily intake of AA based on dietary ingestion was evaluated using the HBM data acquired herein and by applying the methodology previously described in the literature (Fernández et al., 2022). Briefly, GM and P95 of $\sum$UAAM (both adjusted by volume and Cre) in the children under study were transformed to EDIs of AA via the following equations (Heudorf et al., 2009; Mojska et al., 2020):

$$EDI_{Vol}\left(\frac{\mu g\ AA}{kg-bw{\cdot}day}\right) = \frac{\sum UAAM_{Vol} \times V_{24h} \times MW_{AA}}{F_{UE} \times BW} \quad (1)$$

$$EDI_{Cre}\left(\frac{\mu g\ AA}{kg-bw{\cdot}day}\right) = \frac{\sum UAAM_{Cre} \times Cre_{24h} \times MW_{AA}}{F_{UE} \times BW} \quad (2)$$

where $\sum$UAAM = sum of the molar concentrations of AAMA, GAMA3, and AAMA-Sul in urine ($nmol{\cdot}l^{-1}$ for EDI_{Vol} or $nmol{\cdot}g\ Cre^{-1}$ for EDI_{Cre}); V_{24h} = daily volume of urine normally excreted by children (0.66 $l{\cdot}day^{-1}$) (Aylward et al., 2011); Cre_{24h} = daily urinary creatinine excreted by children (0.50 g $Cre{\cdot}day^{-1}$) (Aylward et al., 2011); MW_{AA} = molecular weight of AA (71 g mol^{-1}); F_{UE} = urinary excretion factor of ingested acrylamide which is eliminated as $\sum$UAAM (0.5) (Kocadağli and Gökmen, 2016); and BW = mean body weight of the children under study (33 kg).

2.5. Risk assessment

Interpretation of the risks associated with the AA urinary levels based on the non-neoplastic effects of AA on the target population was performed using two different approaches: reverse and forward dosimetry (Gurusankar et al., 2017). The risk associated with the neoplastic effects of AA was not estimated due to there is no clear evidence of its carcinogenicity in humans (EFSA, 2015).

2.5.1. Reverse dosimetry

In this strategy, the risk was estimated considering only ingestion as the main exposure route as the predominant source of AA exposure in children was food (EFSA, 2015). For this purpose, the margin of exposure (MOE) was calculated as the ratio of the benchmark dose lower confidence limit ($BMDL_{10}$) for neurotoxicity in rats (430 μg AA·kg-$bw^{-1}{\cdot}day^{-1}$) (EFSA, 2015) and EDI (equation (3)).

$$MOE\ (dimensionless) = \frac{BMDL_{10}\left(\frac{\mu g\ AA}{kg-bw{\cdot}day}\right)}{EDI\left(\frac{\mu g\ AA}{kg-bw{\cdot}day}\right)} \quad (3)$$

According to the EFSA's estimation, MOEs lower than 125 may indicate a human health concern because of the non-genotoxic effects of AA on human health (EFSA, 2015).

2.5.2. Forward dosimetry

In the forward dosimetry approach, transformation of the HBM data is not required as the urinary levels of AAMA are directly compared with the non-cancer Biomonitoring Equivalent (BE) and the BE based on the human equivalent point of departure (BE_{POD}) derived for AA (Hays and Aylward, 2008). Consequently, BE and BE_{POD} for urinary AAMA concentrations based on volume (13 and 130 μg l^{-1}, respectively) and Cre levels (16 and 160 μg g Cre^{-1}, respectively) were characterized in prioritization schemes for direct comparison with GM and P95 of the obtained AAMA urinary levels (Hays and Aylward, 2009). These schemes provide an initial estimation of whether the exposure to AA is of low (below BE), medium (between BE and BE_{POD}), or high (above BE_{POD}) concern and priority for further monitoring of risk assessment in the study population group. However, they cannot be used to distinguish "safe" exposures from "unsafe" exposures (Hays and Aylward, 2009, 2008).

3. Results

A total of 612 urine samples were included in this study as the urine volumes of some children (n = 49) were not sufficient to determine AA metabolites and the urinary Cre concentrations in 5 children were abnormal (namely, beyond the 30–300 mg dl^{-1} range) (Barr et al., 2005).

A summary of the sociodemographic characteristics and food consumption habits of the children under study is presented in Table 1. As the urinary biomarkers of AA represent short-term exposure to AA (Hays and Aylward, 2008; Kocadağli and Gökmen, 2016), the information of the 72 h-recall questionnaire was used to characterize and identify potential sources of AA exposure. This included the following food commodities: fried potato products (such as French fries, chips, and other snacks), cereals, bread, and precooked products (EFSA, 2015). According to EFSA, biscuits are also a relevant source of exposure to AA in children (EFSA, 2015), and due to the employment of AA in polymers used in food packaging, the consumption of plastic-packed products was also considered a potential exposure source in the present study (EFSA, 2015). However, information on the consumption of these foods was only included in the food frequency questionnaire and not in the 72 h-recall. Therefore, the intake of biscuits and plastic-packed products

Table 1
Sociodemographic characteristics and food consumption habits of the children participating in the BIOVAL study (n = 612).

Children's characteristics	n (%)	Mean (minimum – maximum)
Body Mass Index (BMI, kg·m^{-2})		18 (12–45)
Missing data	19 (3%)	
Age (years)		8 (5–12)
Province		
Alacant	225 (37%)	
Castelló de la Plana	134 (22%)	
València	253 (41%)	
Home location		
Urban	482 (79%)	
Other	129 (21%)	
Missing data	1 (0.2%)	
Sex		
Male	311 (51%)	
Female	301 (49%)	
Country of birth		
Spain	600 (98%)	
Foreign	9 (1.5%)	
Missing data	3 (0.5%)	
Physical activity		
3 or more days per week	320 (52%)	
1 or 2 days per week	225 (37%)	
Occasionally or never	67 (11%)	
Parents' characteristics	**n (%)**	
Country of birth		
Both parents were Spanish	527 (86%)	
At least one of the parents was foreign	67 (11%)	
Missing data	18 (3%)	
Maximum level of education		
Without studies or primary studies	67 (11%)	
Secondary studies	185 (30%)	
University studies	360 (59%)	
Employment situation		
Both unemployed	39 (6%)	
Only one parent employed	181 (30%)	
Both employed	392 (64%)	
Children's food consumption habits related to AA exposure	**n (%)**	**Mean (minimum – maximum)**
Potato fried products in the last 72 h (g)[a]		26 (0–210)
Cereals in the last 72 h (g)		29 (0–270)
Bread in the last 72 h (g)		80 (0–225)
Precooked products in the last 72 h (g)		3 (0–90)
Missing data	1 (0.2%)	
Monthly consumption of biscuits (g·month^{-1})		1000 (25–4500)
Missing data	7 (1%)	
Monthly consumption of plastic-packed food (number of products·month^{-1})		
Missing data	7 (1%)	6 (0–21)

[a] Including French fries, chips and other snacks.

(including processed and plastic-wrapped foods) in grams per month were considered (Table 1). The complete consumption frequency questionnaire (grams of food group per month) is presented in Table S1.

The study population was a representative sample of 5–12-year-old children living in the Valencian Region (Spain) because the percentage of the children recruited by province was in accordance with the geographical distribution of the Valencian population (Pérez et al., 2017), and both sexes were equally represented (51% males and 49% females). Nevertheless, exposure to secondhand smoke was not recorded in the questionnaire; therefore, its influences on the levels of AA metabolites were not analyzed.

3.1. Urinary concentrations of AA biomarkers

Statistical descriptors of urinary levels of AAMA, AAMA-Sul, and GAMA3 (ng·ml^{-1} and μg·g^{-1} Cre) for the children of the BIOVAL study are presented in Table 2.

Urinary Cre levels were between 33 and 266 mg dl^{-1}, with a mean of 105 mg dl^{-1}. The three AA metabolites were quantified above the LOQ in all urine samples, exhibiting wide AA exposure in children living in the Valencian Region. The major AA biomarker, AAMA, was detected at the highest concentrations, 11–796 ng ml^{-1} (GM = 79 ng ml^{-1}), the levels of its sulfoxide derivative, AAMA-Sul, ranged from 5 to 140 ng ml^{-1} (GM = 28 ng ml^{-1}), and the concentrations of the mercapturic acid of GA (GAMA3) were the lowest, ranging from 2 to 125 ng ml^{-1} (GM = 18 ng ml^{-1}). The mean oxidative conversion of AA to GA (GAMA3/AAMA) was estimated to be 23%.

3.2. Statistical analysis

The Spearman correlation matrix provided in Table S2 shows a high correlation among AAMA, GAMA3, and AAMA-Sul (p-value < 0.01), corroborating that AA is the common source of these mercapturic acids.

A schematic showing the results of the prediction model for log-transformed ∑UAAM is depicted in Fig. 1. The thickness of the lines demonstrates the influence of each significant variable on ∑UAAM. The obtained model explained the 27.5% of the data variability. It should be considered that only children with a completed questionnaire (n = 560) were included in the construction of the prediction model.

According to the regression analysis, Cre concentration had the strongest positive influence on AA metabolite levels. Furthermore, the consumption of fried potato products (including French fries, chips, and other snacks) in the last 72 h and the monthly intake of biscuits were related to higher ∑UAAM.

Regarding the sociodemographic characteristics of the participants, sex was considerably associated with internal exposure to AA; however, only slight differences were found between the mean ∑UAAM of males (694 nmol g Cre^{-1}) and females (621 nmol g Cre $^{-1}$). Another relevant predictor was the age of the children, which showed a negative correlation with AA exposure.

3.3. EDIs and risk assessment

The EDIs calculated based on volume and Cre levels are shown in Fig. 2. Similar EDIs were acquired at GM concentration levels, ranging from 1.2 to 1.5 μg AA·kg-bw^{-1}·day^{-1}. In contrast, substantially higher EDIs were obtained at the P95 distribution levels for volume-based concentrations (4.9 μg AA·kg-bw^{-1}·day^{-1}) as compared to those acquired using Cre levels (3.2 μg AA·kg-bw^{-1}·day^{-1}).

To evaluate these EDIs in a risk assessment context, the MOE concept was applied. Fig. 3 depicts the MOEs obtained using the EDIs adjusted by volume and Cre levels. The mean MOEs ranged from 283 to 364. Only the MOE for P95 based on volume was lower than 125.

Regarding the forward dosimetry approach, risk prioritization schemes were used to interpret the AAMA urinary levels (Fig. 4). The mean concentrations of AAMA were significantly higher than BE,

Table 2
Descriptive statistics of the urinary AA metabolites (UAAM) concentrations in Spanish children (n = 612).

Metabolites	LOQ	DF (%)	Minimum	P25	P50	GM	P75	P95	Maximum	SD	Units
AAMA	0.25	100	11	46	78	79	124	290	796	97	$ng{\cdot}ml^{-1}$
AAMA-Sul	0.25	100	5	18	29	28	45	78	140	22	
GAMA3	0.25	100	2	11	18	18	27	49	125	14	
[a]∑UAAM	–	100	88	321	528	535	818	1719	4013	532	$nmol{\cdot}l^{-1}$
Ratio GAMA3/AAMA	–	–	0.06	0.16	0.24	0.23	0.32	0.46	0.92	0.12	Dimensionless
$AAMA_{Cre}$	–	–	10	51	76	81	121	270	742	84	$\mu g{\cdot}g\ Cre^{-1}$
$AAMA\text{-}Sul_{Cre}$	–	–	5	20	28	29	43	72	170	20	
$GAMA3_{Cre}$	–	–	4	13	18	18	27	46	135	14	
[b]$\sum UAAM_{Cre}$	–	–	78	366	519	549	796	1503	3664	463	$nmol{\cdot}g\ Cre^{-1}$

LOQ: Limit of quantification; DF: Detection Frequency (% samples > LoQ); P: percentile; GM: Geometric mean; SD: Standard deviation; Cre: creatinine.
[a] $\sum UAAM = AAMA + AAMA\text{-}Sul + GAMA3$.
[b] $\sum UAAM_{Cre} = AAMA_{Cre} + AAMA\text{-}Sul_{Cre} + GAMA3_{Cre}$.

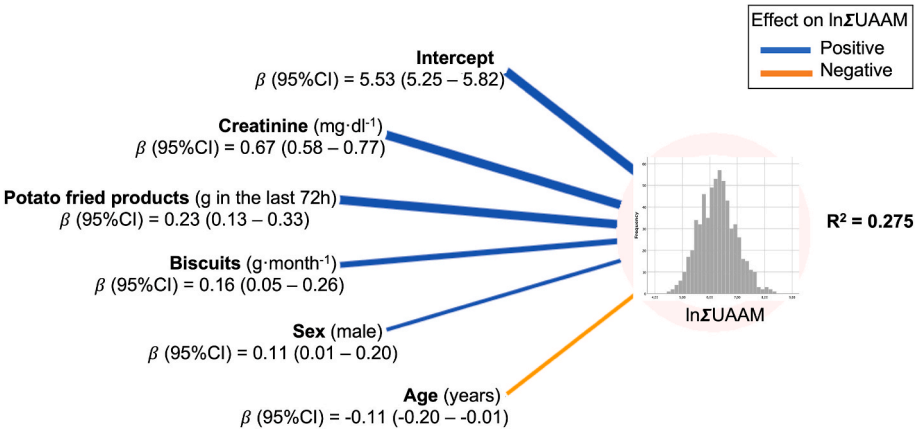

Fig. 1. Linear regression model fitted for the log-transformed ∑UAAM (n = 560, only participants with a completed questionnaire were considered), with its corresponding regression coefficients, 95% confidence intervals and coefficient of determination (R^2). The thickness of the lines represents the influence of each significant variable on ∑UAAM.

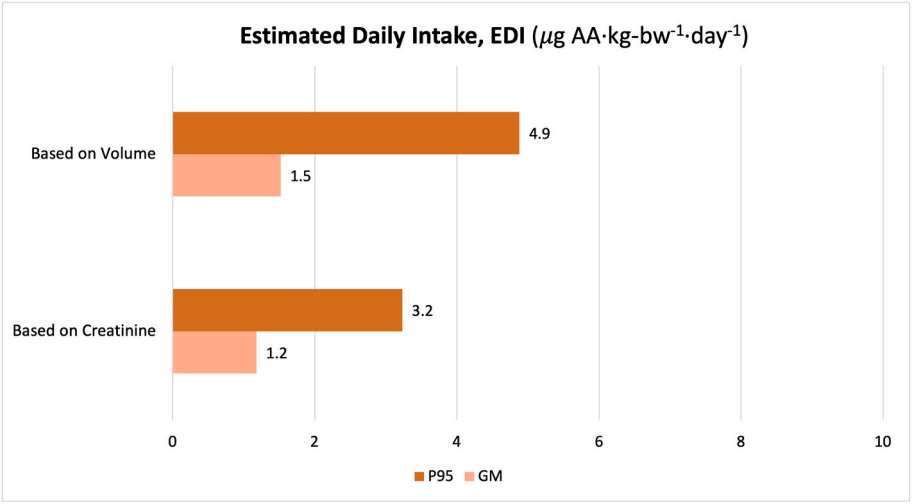

Fig. 2. Estimated daily intakes of AA (EDI, in μg $AA{\cdot}kg\text{-}bw^{-1}{\cdot}day^{-1}$) calculated from ∑UAAM adjusted by volume and by creatinine at GM and P95 distribution levels.

whereas they were substantially lower than BE_{POD}, indicating a medium-priority risk of AA exposure in the children under study. However, the high-priority cut-off value exceeded when P95 was considered.

It is important to note that the use of biomarkers to estimate exposure to food contaminants, such as acrylamide, has advantages and disadvantages. First, biomarkers of exposure provide us a confirmation of the absorption of contaminants by the human body and give information on aggregate and cumulative exposure to the parent compound (Kromerova and Bencko, 2018; WHO, 2011). In addition, the evaluation of biomarker levels helps to understand their toxicokinetic, as well as to identity exposure trends and evaluate public health interventions

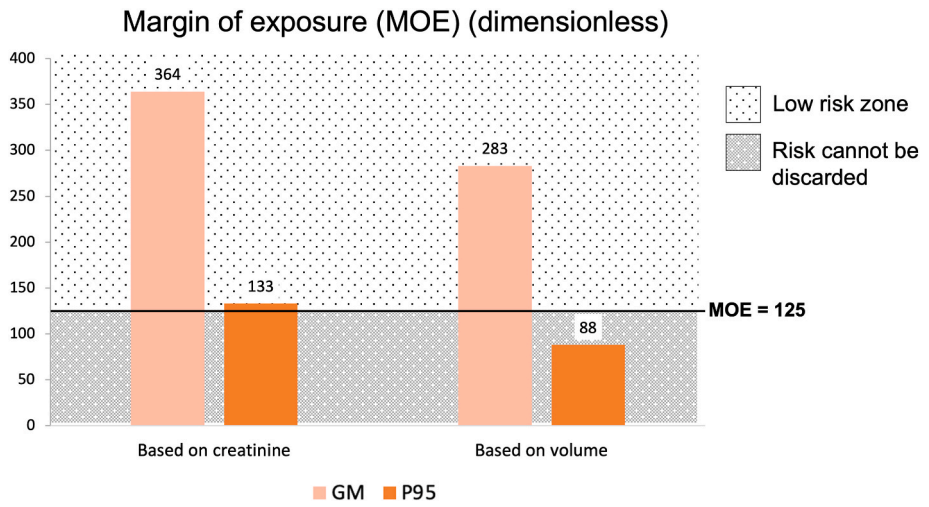

Fig. 3. Results of the risk assessment using reverse dosimetry: representation of the margin of exposure (MOE) at GM and P95 distribution levels adjusted by volume and creatinine.

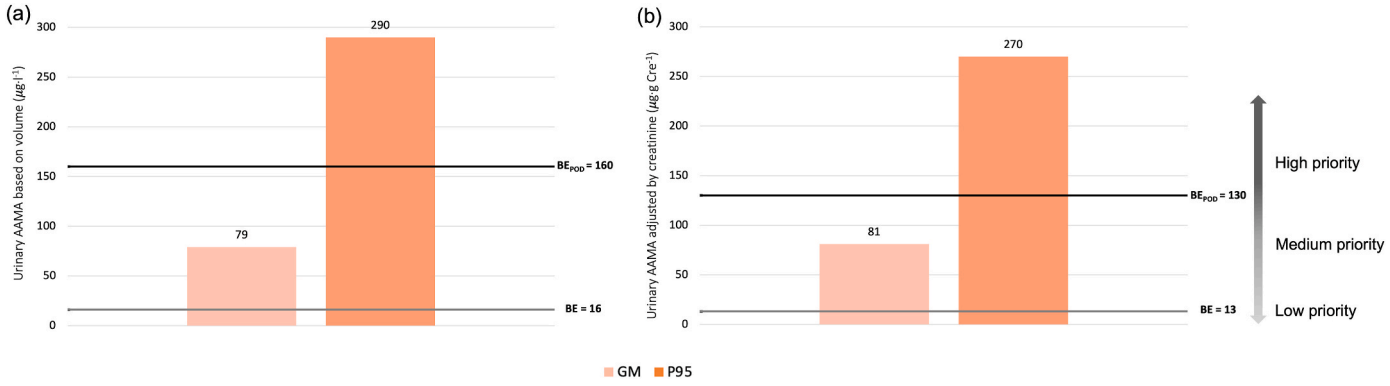

Fig. 4. Results of the risk assessment using forward dosimetry: prioritization diagrams based on biomonitoring equivalents (BEs) at GM and P95 distribution levels adjusted by volume (a) and creatinine (b).

(Kromerova and Bencko, 2018; WHO, 2011). However, biomarkers cannot distinguish among sources, pathways or different durations of exposure (WHO, 2011). Furthermore, health-based reference values in HBM, such as BEs or HBM-I and –II, are still lacking for most food contaminants, and they do not represent diagnostic criteria (safe vs unsafe exposure). In the case of acrylamide, BEs were derived with a "medium" confidence (Hays and Aylward, 2008).

4. Discussion

Herein, the urinary levels of AAMA, GAMA3, and AAMA-Sul were measured in children living in the Valencian Region (Spain) to evaluate the levels of AA exposure and the risk associated with AA exposure. Due to the representativeness of the sample, these data may be used as reference values for this subpopulation group in the Eastern Spanish region.

The levels of the mercapturic acid metabolites of AA in urine were in the following order: AAMA > AAMA-Sul > GAMA3. This is in accordance with the metabolic and toxicokinetic properties of AA in humans (Kocadağli and Gökmen, 2016) and the abundances observed in two similar HBM studies (Brisson et al., 2014; Fernández et al., 2022). Additionally, the high detection frequencies acquired in this study (100%) are similar to those reported by other authors (Brisson et al., 2014; Choi et al., 2019; Hartmann et al., 2008; Ji et al., 2013; Kuang et al., 2021; Schwedler et al., 2021; Song et al., 2022), indicating widespread AA exposure in children from different parts of the world.

Only few studies have been reported on the urinary levels of AA biomarkers in children. As many of these studies do not provide GM values, P50 of urinary AA levels was used to compare among populations. Fig. 5 shows a comparison between the P50 levels of urinary AAMA, AAMA-Sul, and GAMA3 acquired in ten studies focused on the population group investigated herein. A more detailed description of each study is provided in Table S3. It should be noted that AAMA-Sul is not usually analyzed in many HBM studies, probably because is a minor metabolite of AA. Most authors only report urinary levels of AAMA (major metabolite of AA) and GAMA3 (major metabolite of GA) (see Table S3).

The median AAMA levels in children involved in the BIOVAL study (76 μg g Cre^{-1}) were comparable to those in children and adolescents from Canada (76 μg g Cre^{-1}) (Brisson et al., 2014) and Korea (79 μg g Cre^{-1}) (Ji et al., 2013), whereas they were considerably higher than those in the rest of the studies (21–59 μg g Cre^{-1}) (Fig. 5). In contrast, the urinary concentrations of GAMA3 were very similar to those observed in children living in Germany (Hartmann et al., 2008; Heudorf et al., 2009; Schwedler et al., 2021), Canada (Brisson et al., 2014), the USA (CDC, 2021), Korea (Choi et al., 2019; Ji et al., 2013), and China (Kuang et al., 2021); nevertheless, the urinary levels of GAMA3 in young Chinese children (0–7 years old) were the lowest (3 μg l^{-1}, adjusted by specific gravity) (Song et al., 2022). In addition to the present study, only one study has been reported on the urinary levels of AAMA-Sul in Canadian adolescents (10–17 years old) (39 μg g Cre^{-1}) (Brisson et al., 2014), which are higher than the concentrations obtained herein (29 μg

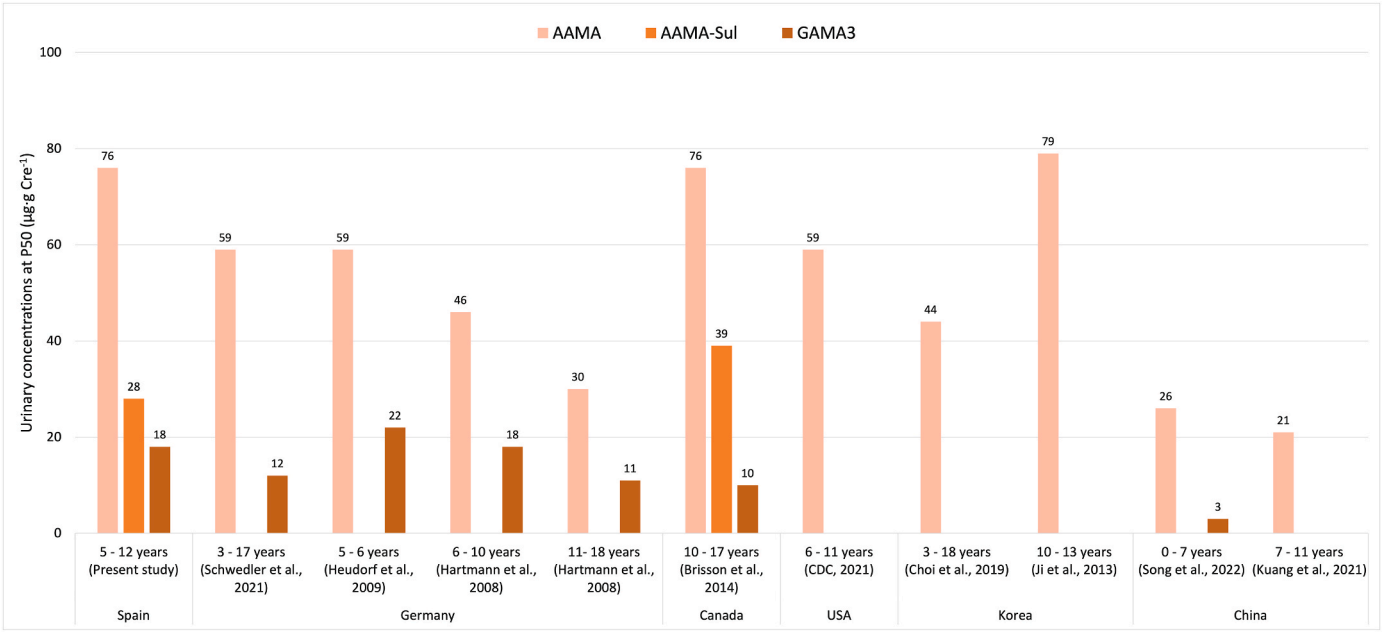

Fig. 5. Comparison of existing HBM data of urinary AA metabolites in children and adolescents from different countries. A detailed description of each study can be found in Table S2.

g Cre^{-1}). The AAMA and GAMA3 concentrations acquired herein for the evaluated children were slightly lower than those recently reported for adult women living in the Valencian Region (Fernández et al., 2022).

Note that this variability in the urinary levels of AA metabolites among children from different nationalities could be related to differences in not only their lifestyles, genetic factors, secondhand smoke exposure, dietary patterns, and cooking traditions, but also the urine sample type, collection year, age range, and the analytical methods used (Choi et al., 2019).

Identifying the sources of AA exposure in humans is crucial for the risk assessment of AA exposure and the development of mitigation and control strategies, specifically for food safety. Therefore, associations between ∑UAAM and some characteristics and dietary habits of the target population were evaluated. Consequently, the effect of diet on AA exposure was confirmed. The urine samples of children who more regularly consumed higher quantities of fried potato products in the last 72 h and biscuits had significantly higher ∑UAAM than those in the case of the children who consumed less quantity of these food items. In fact, EFSA estimates that 51 and 25% of the dietary AA exposure in infants, toddlers, and other children are owing to the ingestion of fried potato products and biscuits (together with other bakery products), respectively (EFSA, 2015). Similar tendencies have been observed in other HBM studies, in which fried potatoes (Choi et al., 2019; Heudorf et al., 2009; Schwedler et al., 2021) and bakery products (including biscuits) (Heudorf et al., 2009; Lee et al., 2013) were found to be relevant contributors to the internal exposure of this population group to AA.

With respect to sociodemographic characteristics, higher ∑UAAM was obtained for children in lower age ranges, implying an association between age and AA internal exposure. This finding was in agreement with the observed mean ∑UAAM of 723, 685, 629, and 593 nmol g Cre^{-1} for 5–6-, 7–8-, 9–10-, and 11–12-year-old children, respectively. Similar findings have been obtained in other studies (Hartmann et al., 2008; Schwedler et al., 2021), confirming that younger children are more vulnerable to AA exposure as they consume more food, inhale more air, and drink more water per kg-bw than those in the cases of older children (Mielech et al., 2021). Gender-related differences were also noticed: males demonstrated higher urinary levels of AA metabolites as compared to those of females. However, the relationship between internal AA burden and sex is controversial as some authors have claimed that urinary Çre levels may distort this relationship due to the lower Cre excretion of females comparing to males (Barr et al., 2005; Hartmann et al., 2008; Ji et al., 2013).

To the best of our knowledge, this is the first study on the estimation of AA intake among Spanish children based on HBM data. The mean EDIs derived herein are in the range of those calculated by EFSA for infants, toddlers, and other children in Europe (0.5–1.9 µg AA·kg-bw^{-1}·day^{-1}). In contrast, when P95 was considered, EDIs were close to or exceeded the maximum upper limit reported by EFSA (3.4 µg AA·kg-bw^{-1}·day^{-1}) (EFSA, 2015). In a diet study based on the food consumption habits of Spanish children (5–14 years old) in 2013, lower EDIs were acquired (≈0.90 µg AA·kg-bw^{-1}·day^{-1}) (Molina Périz et al., 2016). These differences might be due to the fact that the HBM data take into account not only ingestion of AA, but also other pathways of AA exposure, such as inhalation of secondhand smoke (Kuang et al., 2021) and dermal contact with consumer products (WHO, 2002). Additionally, endogenous generation of AA in the human body can be a potential contributor to internal AA exposure (Rietjens et al., 2018). Compared to our previous study, herein, comparable EDIs were obtained for women living in the same region (Fernández et al., 2022). Nevertheless, other HBM studies focused on children from Korea (Ji et al., 2013) and Germany (Hartmann et al., 2008; Heudorf et al., 2009) demonstrated lower mean EDIs (1.04 and ≤ 1.13 µg AA·kg-bw^{-1}·day^{-1}, respectively) than those found for the population under study. Note that these studies did not include the urinary levels of AAMA-Sul (both AAMA-Sul and GAMA3 were not considered in the Korean study) in the daily intake calculations, which could lead to lower robustness of the EDI estimations.

According to the EFSA CONTAM Panel, current levels of dietary AA exposure in infants, toddlers, and other children are not of concern with respect to the non-neoplastic effects of AA as MOEs were estimated at mean levels ranging from 860 to 226 and from 307 to 126 in P95 (EFSA, 2015). However, although the MOEs at GM level acquired herein were in the same safe range, MOEs at P95 were close to or below 125, revealing a potential health risk of AA in children living in Spain. Additionally, the urinary AAMA levels found in the BIOVAL study were up to six orders of magnitude higher than the corresponding BE, indicating that risk assessment of AA should be performed. This finding was also acquired for children living in the other abovementioned countries (Fig. 5).

5. Limitations and strengths of the study

The major limitation of the study was the lack of information about exposure to second-hand smoke, consumption of specific foods, such as breaded food, and culinary practices of the study population, which may have a big influence on urinary levels of AA metabolites. In addition, the inter-individual variability in the metabolism of children was considered as their creatinine concentration in urine, which may not be the best surrogate estimator since its excretion is influenced by physiological and exogenous factors. Moreover, spot urine samples were used for the exposure estimation, which give us a less certain estimate of long-term exposure than 24-h samples.

On the other hand, a major strength of the study was that it was conducted using a representative sample of the infant population living in the Valencian Region. Thus, the sample size of the study together with the high detection frequencies observed for the three main metabolites of AA led to a high statistical power of the results. Additionally, the first HBM data on AA exposure in Spanish children were presented, highly contributing to the knowledge of AA exposure in this country.

6. Conclusions

Herein, the urinary concentrations of AA metabolites in children (5–12 years old) living in Spain were analyzed for the first time. The consumption of fried potato products and biscuits was an important contributor to internal AA exposure in this population group. Considering the potential negative effects of AA, its high frequency of detection in urine, and the higher concentrations of AA metabolites than the BE, it is concluded that AA may pose a health risk for children and should be listed as a priority for risk assessment and management activities in Spain and other countries.

In conclusion, further HBM studies on the internal body burden of AA are necessary to characterize and monitor the potential risks and sources of AA in children and to provide more data to food safety authorities to facilitate mitigation measures for reducing dietary exposure to AA. In addition, the need of reducing the consumption of foodstuffs with a high content of AA, such as fried potato products, in Spain has been evidenced and should be recommended to consumers by Spanish public health authorities with more effectiveness.

Author statement

Sandra F. Fernández: Methodology, Validation, Investigation, Writing – original draft, Visualization, Software, Formal analysis, Data curation; **Olga Pardo:** Writing – review & editing, Supervision, Funding acquisition; **Clara Coscollà;:** Supervision, Project administration; **Vicent Yusà:** Conceptualization, Writing – review & editing, Supervision, Project administration.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119319.

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