Pérez-Torres, R.; Doménech Antich, EM.; Conchado Peiró, A.; Sanchez, A.; Coscollà, C.; Yusà, V. (2018). Influence of diet in urinary levels of metals in a biomonitoring study of a child population of the Valencian region (Spain). The Science of The Total Environment. 618:1647-1657. https://doi.org/10.1016/j.scitotenv.2017.10.011

Influence of diet in urinary levels of metals in a Biomonitoring study of a child population of the Valencian region (Spain)

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Abstract

Pollution by trace elements and its possible effect on organisms has become a worldwide concern due to the increasing presence of trace elements in the environment and especially in the food chain. Exposure to chemicals has traditionally been measured using environmental samples, however, human biomonitoring brings a different perspective, in which all sources and exposure pathways are integrated. The objective of this paper is to discern the possible relationship between children's diets and the metals found in their urine. With this aim in mind, a total of 120 voluntaries participated in a diet survey carried out in a school-aged population (age 6-11) from the Valencian region. In addition, twenty trace elements were analysed in their urine (arsenic, antimony, barium, beryllium, caesium, cadmium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, platinum, selenium, thallium, thorium, uranium, vanadium and zinc). Results permitted to compare metal levels in urine with metals levels of other biomonitoring studies to conclude that values, including ours, were similar in most studies. On the other hand, children who ate more vegetables had the highest values in cadmium, copper, molybdenum, antimony, thallium, vanadium, and zinc, while those who ate more fish reached higher values in mercury. Finally, children who ate more cereals and baked products got higher values in total arsenic.

Keywords: Biomonitoring; children; metals; urine

1. Introduction

Elements are natural occurring chemical compounds, so they can be present at various concentrations in the environment. However, industrial, domestic, agricultural, medical and technological applications increase their presence above their natural level, contaminating not only water, air and soil, but also crops and animals. For this reason, the food chain is one of the most important pathways of exposure to metals for general population (Kachenko and Singh, 2006; Sharma et al., 2007). A significant number of studies have surveyed metals in food. Various studies have recently been published on vegetables (Noli, &Tsamos, 2016; Shaheen et al.,2016; Li et al., 2017, etc); on fish (Saha et al., 2016; Makedonski et al., 2017; Gu et al., 2017); water (Chowdhury et al., 2016); dairy products (Shahbazi et al., 2016; Suturović et al., 2014); or cereals, (Akinyele, &Shokunbi, 2015; Cuadrado et al., 2000).

Element concentrations in foodstuffs and their associated population health risks have drawn increased attention, globally. Not only the considered toxic elements such as mercury, lead, cadmium, and arsenic (Valko et al., 2006; Brewer, 2010 Annangi et al., 2016) can produce adverse effects, but those elements considered essential for physiological or vital functions, can also cause several clinical and physiological problems when in excess (WHO, 1996).

In recent years, there has been an increasing susceptibility to various diseases such as diabetes, cardiovascular disease, cancer, mutagenicity, and teratogenicity after a prolonged exposure to some trace elements (Kakkar and Jaffery, 2005; Landrigan and Etzel, 2014; Korashy et al., 2017). This may be due to elements that accumulated in certain tissues over a long period of time until the element reaches a critical level in the body that leads to illness or, alternatively, there could be a long latent period between exposure and first symptoms of the disease (Järup, 2003; Zhai et al., 2017).

Human biomonitoring (HBM) can be defined as “the method for assessing human exposure to chemicals or their effect by measuring these chemicals, their metabolites or reaction products in human specimens” (CDC, 2005). One of the main advantages of the HBM approach is that it represents an integration of exposure from all sources and routes, providing an important perspective on overall exposure and making HBM an ideal instrument for risk assessment and risk management. In addition, it permits to determine the level of exposure in the general population, identifying vulnerable groups and highly-exposed populations. Also, when data are extended over a period of time HBM studies can identify trends, changes or even new chemical exposures (Albertini et al., 2006; Angerer et al., 2007; Wittassek et al., 2011).

The objective of this paper is to discern the possible relationship between children’s diets and metals found in their urine. With this aim in mind, a total of 120 volunteers from the Valencian region, between six and eleven years of age, participated in a cross-sectional study, which consisted of two parts: 1) a diet survey and 2) a biomonitoring study. Herein, twenty trace elements were analysed in the children’s urine (arsenic, antimony, barium, beryllium, caesium, cadmium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, platinum, selenium, thallium, thorium, uranium, vanadium and zinc). Finally, a statistical analysis was made to determine possible associations between the consumption of a specific type of food and the exposure to metals.

2. Material and methods

2.1. Target population

The present study is focused on children aged between 6 and 11. Thus, children were recruited from two public Primary schools, whose directive board was interested to collaborate. To find out whether the school environs and life style had a possible influence, two very different places were chosen. On the one hand, an urban area represented by Valencia (with around 790,200 inhabitants in 2016) and on the other hand, a high agricultural area with an important production of citrus, denominated in this paper as "rural", represented by Alzira (around 44,500 inhabitants in 2016). Finally, a total of 58 volunteer from the Valencia school and 62 from the Alzira school, agreed to participate in the cross-sectional study, which consisted of two parts: 1) a survey and 2) a biomonitoring study. The questionnaires and the study were approved by the Scientific Ethics Committee of the Foundation for the Promotion of Health and Biomedical Research in the Valencian Region (FISABIO).

2.2. Recruitment process and informed consent form

A meeting, in both schools, was conducted to carry out the recruitment process. In which parents were informed about the aims of the study and the implications of participating. At the end of the meeting, the parents who wanted to participate signed a consent form, whereby they accepted to participate in the survey and in the biological sample collection (urine), approving a possible storage of any remaining urine surplus (FISABIO, 2017). The consent form included information on the Biobank for Biomedical Research in Public Health of the Community of Valencia (IBSP-CV), which is part of the Valencia Biobank Network and the National Biobank Platform. In addition, parents were informed that all information obtained from the study would be managed in accordance with the current national and international standards on data protection and by no means would a publication include personal details.

2.3. Instructions to families

Families, who signed the consent and agreed to participate, received a questionnaire to complete at home and a kit (disposable gloves and a sterile 100 ml polypropylene bottle) with instructions on how to collect the first-spot morning urine sample of their child. In addition, they were informed about which day they had to take the fulfilled questionnaire and the urine sample to school, where staff from the public health centre would collect both things.

2.4. Description of the survey

The questionnaire was carefully designed by staff of the Public Health Department, taking as reference previous studies such as ENCV, (2010) and WHO, (2015). Afterwards, it was validated by 12 fathers/mothers who worked in the Public Health Department but who had no relationship with the topic. The number of participants was defined in accordance to the general recommendation to use small groups from 12 to 30 (Anderson, J.C., & Gerbin, D.W. 1991; Hunt, S.D., et al, 1982). The aim of the validation was to confirm the clarity of the instructions and the comprehensibility of the questions, making an exhaustive identification of all response options and estimating the average time required to answer all the questions (Downing, 2006; Downing & Haladyna, 2006; Schmeiser & Welch, 2006; Wilson, 2005).

The final questionnaire, (available in FISABIO), lasted approximately half an hour to be completed and had three sections: a) Social-demographic, where the family was asked about general information about the child, such as age, weight, sex, height, leisure activities, as well as sociodemographic information about the parents such as studies and work; b) Weekly food consumption of children, a total of 100 questions were asked about consumption of different food groups, i.e. dairy products, meat, fish and eggs, vegetables, fruits, beans and cereals, oil and fats, baked goods and others. In cases which children were eating at school, the menu information was also considered. c) Environmental exposure. A total of nine main questions with some sub-questions were asked, such as smoking habits of the parents, whether the house had a backyard or the family lived near a park. Also, if whether they had pets and if so, about possible pets' treatment in the last month, etc.

2.5. *Biomonitoring sample collection*

The sampling collection was made from May to June of 2011. The specified days parents delivered a urine sample of their child to school, corresponding to the first morning urine of that same day. Samples were collected by the Health Centre team following the international guides and the Expert Team to Support the Biomonitoring in Europe Protocol for sample collection (ESBIO, 2004).

For the next stage, in particular two conditions were established: transport refrigeration (samples were keep below 4ºC) and transport time (transport should last less than two hours). Once in the Biobank for Biomedical Research and Public Health of the Valencian Community (Spain), urine samples were stored at -80ºC until analysis.

2.6. *Target elements*

Twenty elements were analysed including those considered essential such as copper (Cu), cobalt (Co), manganese (Mn), molybdenum (Mo), vanadium (V) and zinc (Zn), and those considered moderately or highly toxic such as arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), caesium (Cs), nickel (Ni), lead (Pb), platinum (Pt), antimony (Sb), selenium (Se), thorium (Th), thallium (TI), uranium (U) and mercury (Hg) (WHO, 1996). In order to discuss the possible health consequences, the measured elements in this paper were grouped according to the classification of the International Agency for Research on Cancer (IARC, 2017). Thus, three groups were considered: 1) Not classifiable as to its carcinogenicity to humans (Group 3) and elements not classified by IARC; 2) High toxicity, possibly and probably carcinogenic (group 2A and group 2B) and 3) Carcinogenic to humans (Group 1). In order to compare our results with previous HBM studies, this paper references other studies, which were selected taking into account three criteria: 1) researches with the same metals and matrix 2) HBM studies made later than 2000, as exposure to environmental chemicals in the population can vary over time; 3) Children and if it is possible the same age group, excluding studies made on adult groups.

2.7. *Chemical analysis*

Chemical analysis was performed in the Public Health Laboratory of Alicante (Spain) following the methods previously described by Roca et al., (2016). Table 1 shows de analytical technique and the Limit of Quantification (LOQ) of the elements determined.

Briefly, the analysis of metals by ICP-MS, mercury and creatinine, were performed as follows

2.7.1 Metals determination by ICP-MS

Except for Hg, analysis of metals were performed on an ELAN DRC II ICP-MS PerkinElmer (Waltham, Massachusetts, USA) equipped with a PFA Standard concentric nebulizer and a Peltier cooled (PC3) baffled glass cyclonic spray chamber. The ICP-MS instrumental operating conditions are shown in Table SI-1 (Supplementary information document). The isotopes measured, the instrumental mode and the DRC conditions for each element are described in Tables SI-2 and SI-3 (Supplementary information document).

Urine vessels were sterile and were not cleaned to eliminate background contamination. However, background concentration of metals was tested and blanks were used in each sequence. The analytical sequence followed in each batch of samples is described in Table SI-4 (Supplementary information document). As a routine basis, several performance parameters (i.e., sensitivity in the whole mass range, reading precision, double-charges and oxide formation and detector background signal) were checked daily with the 1 µg/L tuning solution. If performance requirements were not fulfilled, the optimisation of some instrumental parameters, such ion lens voltage, nebuliser flow rate and detector voltages was carried out in order to maximise and stabilise ion signals and to minimise interference effects from polyatomic and doubly-charged ions. Urine contains high levels of dissolved solids (Urea and uric acid, creatinine, bicarbonates, chlorides, sodium and potassium ions) which can cause both spectral and matrix-induced interferences on the analytes of interest. Therefore, accurate element determinations in this matrix can be difficult. An example of polyatomic species that interfere with analyte isotopes are shown in Table SI-5 (Supplementary information document). Therefore, in order to eliminate or reduce the impact of these interferences, the capabilities of the dynamic reaction cell technology (DRC) were used.

Two methods of analysis were used. The first one combines the determination of Co, Cu, Mn, Ni, Se and Zn in DRC mode using methane (CH4) as a reaction gas together with the rest of analytes (Ba, Be, Cd, Cs, Mo, Pb, Pt, Sb, Th, Tl and U) analysed in Standard mode. The second one uses the DRC’s ability to move some analytes (As and V) to a new analytical mass, away from the interferences. This is achieved using oxygen (O2) as a reaction gas and takes advantage of the rapid reaction between As+ and V+ with O2 to form 75As16O+ at m/z 91 and 51V16O+ at m/z 67, respectively. In this last case, as an internal standard for V, a polyatomic species of Nb (93Nb16O+) was used instead of the single isotope of Nb (93Nb) for better matrix effects and signal drift compensation.

2.7.2 Mercury determination

Mercury was analysed by means of an elemental mercury analyser, DMA-80 Tricell, from Milestone (Sorisole, BG, Italy). The elemental mercury analyser, also known as automated or direct mercury analyser, is a single purpose atomic absorption spectrophotometer for mercury determination. This analyser is based on a sample drying and subsequent thermal decomposition, followed by an electro-thermal atomisation of mercury. A gold amalgamator selectively traps and pre-concentrates the mercury from the flow of decomposition products. Finally, the trapped mercury is released by temperature and detected by atomic absorption at 253.7nm. The instrumental conditions are shown in Table SI-6 (Supplementary information document).

2.7.3 Internal quality control (QC) and method performance

The methodology includes the use of quality control samples (QCS). Five different QCS were chosen to monitor the analytical sequence: Initial Calibration Verification (ICV), Initial Calibration Blank (ICB), Reagent Blanks, Certified Reference Materials (CRM) and Continuous Calibration Verification (CCV) as well as internal standard signal monitoring. When acceptance criteria were not met for a quality control, samples results were discarded, the problem solved (i.e., recalibrating the instrument) and the samples out of control were re-analysed. The limits of detection (LOD) and quantification (LOQ) are presented in Table SI-7 (Supplementary information document) and Table 1. Results for Reference Materials are shown in Table SI-6.

2.7.4. Determination of creatinine

Creatinine measurements were performed to normalize metabolite levels, given the various hydration states of each participant at the time of sampling, due to different dilutions of spot urine samples. Creatinine measurements were done according to the kinetic methodology based on the Jaffé alkaline picrate reaction (Larsen, 1972) using an Architect c16000 automatic analyzer (Abbot Diagnostics, Illinois, USA). Analyses were conducted at the Hospital Doctor Peset in Valencia (Spain).

2.8 Statistical analysis

Chi-square tests and Pearson’s correlation coefficients were performed to identify whether there was any significant relationships between diet and urine metal analysis. Percentages of responses in each category of demographic characteristics were computed, whereas food consumption was graphically represented in a box & whiskers plot. The boxes represent the 25th, 50th (middle line) and 75th percentile, while whiskers represent the range of data (minimum and maximum). When outliers are present, an individual mark (point) is used for identification. All analyses were performed using PASW version 18 software, and SPSS version 17.0 for windows (SPS Inc, Chicago, Illinois, USA)

3 Results and discussion

3.1 Food survey

Demographic characteristics and categories of the participants are listed in Table 2. A total of 120 volunteers were recruited, 62 from Alzira and 58 from Valencia, all of them aged between 6-11 and divided into three subgroups i.e. 6-7; 8-9 and 10-11. Female participation was slightly higher among children in Alzira, whereas similar average weights were observed between children from both locations. Interestingly, Table 2 revealed a significant association between parent's level of education and the location of the family home. The percentage of parents (both father and mother) with university education was higher among those families living in Valencia. We also found particular evidence for a more extended sedentary lifestyle among children in Alzira, as well as a greater percentage of family homes located close to farming areas in this city. Regarding other components of the family home, our findings showed no significant relationship between location and having garden, pets, or even smoking adults at home. Finally, differences were exclusively significant for the use of products aimed to remove lice in children. On average, we found a higher percentage of parents who used these products in Valencia during the month previous to answering. This was maybe due to a head lice outbreak in the school.

The questionnaire shows that the higher number of serving consumed per week (s/w) corresponds to white bread (around 12 s/w), followed by biscuits (8 s/w) and cold(deli)-meat and cheese, both with approximately 6 s/w. On the contrary, the least consumed products are margarine (0.38 s/w), cabbage (0.32 s/w), beans (0.26 s/w), peas (0.24 s/w), avocado (0.21 s/w) and rabbit (0.17 s/w), all of them expressed in servings per week as well. Moreover, 77.6% of participants reported that they usually consumed between two and four eggs a week, and 84.0% reported consuming two or more servings of fish a week. In addition, most children (80%) hade one or two servings of fruit a week.

Taking into account the size of the serving, i.e. the grams consumed per serving (Carbajal and Sánchez-Muniz 2003), and the number of servings eaten in a week, cereals (including baked products) and legumes were again the most consumed groups (mean 988.87±103.25 g/week), being bread and rice the most important products inside these groups. The second most-consumed group was meat and meat products (mean 266.83±283.01g/week), being chicken and pork the most ingested. The third most-consumed group was fruit (mean 191.19± 206.10g/week), where oranges, bananas and apples were the most eaten products. Finally, milk and dairy products (mean 177.85±164.51 g/week), where the highest contribution came from yoghurt and milk. Vegetables are also an important group, which had a huge variability (mean 117.31±837.69 g/week), herein, potatoes and tomatoes were the most consumed. In relation to fish, white fish was preferred, followed by other seafood. Differences based on age, location and sex were not significant (p-value >0.05).

Finally, Figure 1 displays a box and whisker plot containing all the food included in the questionnaire individually (y-axis) and individual food total consumption in one week (x-axis) expressed in grams. Results show that the most consumed products are chicken (mean 813.9g), bottle juice (mean 768.9g), yoghurt (mean 485.7g), oranges (mean 445.2g) pork meat (mean 411.1g), and bread (353.9g).

3.2 Biomonitoring study

Table 3 shows the obtained concentrations of Ba, Cs, Cu, Mn, Mo, Pt, Se, Tl, U and Zn in µg/g creatinine in both locations, i.e. Alzira and Valencia as well as the results of previous studies. Results show that elements had similar values in both locations (p-value >0.05). However, when comparing urine concentration per age, significant differences were found in Cs, Cu, Mo and Se. In all cases a down trend was observed.

Similar results for Cu, Mo and Se were found by Heitland and Köster, (2006). Protano et al., (2016) who analysed trace elements in urine samples from children in a rural area found significant dependency of Cu to age and gender finding than females present higher values. CuAlso, Aguilera et al., (2010) found slightly higher concentrations of Cu with age, justifying this fact with differences in dietary patterns and/or gastrointestinal absorption between children and adults. In addition, in case in which parents smoked significant differences were observed for Ba (p-values 0.0026). Our results do not show a relationship between Mn and children, which parents smoke, however, Protano et al., (2016) concluded that the environmental tobacco smoke, is one of the most important environmental risks for human health and observed a significantly increased in the median levels of Mn (0.74 µg/L for exposed group vs. 0.60 µg/L for unexposed one.

Comparing our results with earlier HBM studies (Heitland & Koster, 2006; Aguilera et al., 2010; Protano et al., 2016; CDC, 2017), Cs, Mo, Pt, Tl and U show similar values. On the other hand, the rest of elements were found in higher values in geometrical mean as well as 50th, 90th and in most of the cases at 95th percentiles. It is worth highlighting that most of the elements considered in Table 3 are essential for health; however, over-exposure can cause several clinical disorders. For example, high levels of Zn can have a gastrointestinal effect, and Mn and Se can result in neurological effects.

Table 4 shows the obtained urinary levels (µg/g creatinine) of possibly and probably carcinogenic elements (Be, Co, Hg, Pb, Sb and V) and includes findings from other authors (Heitland & Koster, 2006; Molina-Villalba et al., 2015; CDC, 2017). Results indicate that non-significant differences exist between the locations of Alzira and Valencia in relation to Pb, Sb and V. On the contrary, differences can be observed for Co and Hg. Moreover, taking into account the age, significant differences were observed in V (p-value 0.0013). The same result was obtained by Heitland & Koster, 2006 who observed that among children, urinary concentrations of these elements decrease with age. On the other hand, V and Hg in urine are associated with recent exposure since they rise within a few hours after the onset of exposure (Alimonti et al., 2000; Järup, 2003).

Comparing our results with previous HBM studies, Sb, had slightly higher levels. While our children population had a 50th percentile of Pb (1.04 and 1.25 µg/g creatinine in Alzira and Valencia, respectively). Theses values are lower than those published by Molina-Villalba et al., (2015). Regarding V, our findings are similar to earlier HBM findings, in which the same age group was being studied (Heitland & Koster, 2006). .

Urine concentration of Co is highly related to the area, however for non-polluted areas, Co exposure is related to food sources, including cobalamin i.e. vitamin B-12 (Banza et al., 2009). Findings on Co levels in our child population (GM= 1.27 and 1.74 in Alzira and Valencia, respectively) were higher than reported by other authors. Nevertheless, the obtained levels (1.47 and 1.39 µg/L in both locations) were very far from the Biological Exposure Index (BEI) of 15 µg/L suggested by the American Conference of Governmental Industrial Hygienists (ACGIH, 2006).

Mercury is known to be neurotoxic to humans and children are especially vulnerable to this exposure, and there is a huge concern that pregnant women and children with high fish consumption may impair child development (Llop et al. 2014). However, Hg presence in urine is associated to recent exposure to inorganic mercury compounds (less toxic) (Järup, 2003). When comparing our results with other studies, our findings were higher than Health Canada (2015) and CDC, (2017). On the other hand, our results were lower than those reported by Molina-Villalba et al., 2015. The HBM Commission has defined two thresholds for Hg: HBM-I= 5µg/g creatinine and HBM-II= 20 µg/

creatinine. Where, the HBM-I-value represents the concentration of a substance in human biological material below which, there is no risk of adverse health effects and consequently no need for action. However, when the value obtained is higher than the HBM-I but lower than the HBM-II the result should be verified by further measurements. Urine concentration of Hg obtained is below the HBM-I value even at the 95th percentile (2.68 and 2.79µg/g creatinine) and maximum value (3.91 and 3.81 5µg/g creatinine) in Alzira and Valencia, respectively. Therefore, no further actions should be taken.

Finally, although Be is considered to be inside this group (possibly and probably carcinogenic elements), it was not included because it was not found in urine samples. This is in agreement with earlier studies (Heitland & Koster, 2006 ATSDR, 2014; Bas et al., 2016; Protano et al., 2016), which remark Be cosmic origin and low presence in air, food and water.

Table 5 shows the concentrations for the rest of carcinogenic elements that have been analysed in this paper i.e. As, Be, Cd, Ni and Th. Arsenic is widely distributed throughout the environment via air, soil, water and most plant and animal tissues due to the high mobility of its chemical compounds (Cook et al., 2005; Mandal & Suzuki, 2002). For this reason, As usually reaches the human body through plants that have been irrigated with contaminated groundwater (Meharg et al., 2008; Panaullah et al., 2009). Results do not show significant differences between locations, nor age or familiy life-style. The geometric mean values of As (29.6 and 38.45 µg/g creatinine in Alzira and Valencia, respectively) found in our study are remarkably higher than other values reported (Heitland & Koster, 2006). However, they were similar or lower than those reported by Carrizales et al. (2006) (46µg/g creatinine) and Jasso-Pineda et al. (2012) whose mean reported value was 24.7µg/g creatinine, but peaked at 44.5µg/g creatinine in one of the communities studied. Overall, our child population had As concentrations within the normal concentration of 100 µg/L accepted by the ATSDR (2007). In addition, in relation to possible health consequences, the present paper assessed the total As (including organic and inorganic As). However, only inorganic arsenic (i-As) is classified as group 1 carcinogenic (IARC, 2017). On the other hand, organic arsenic species (o-As) are thought to have lower toxicity (Mass et al. 2001; Styblo et al., 2000).

Regarding Cd, it is a non-essential toxic metal that is widely distributed at low levels in the environment. The main pathway of human exposure is cigarette smoking and food for non-smokers (especially pulses, tubers and cereals). The geometric mean of Cd found in the present work is around 0.20 µg/g creatinine, which is very similar to what most of authors reported (Table 5) and lower than the 0.41 and 0.0.53µg/g creatinine reported by Aguilera et al. (2010) in two different areas. However, these data are higher than the exposure values in children found in Spain (GM=0.047) and in European (GM=0.071), through the programme COPHES/DEMOCOPHES study, both of them reported by Den Hond et al. (2015). Nevertheless, even the maximum values found (0.98 and 0.42 µg/g creatinine) in two locations studied seem to be correct as only values of urine Cd above 1 µg/g creatinine has been associated with renal tubular dysfunction, calcium metabolism disturbances and a higher risk of lung cancer (Banza et al., 2009). In addition, all values even at the 95th percentile (0.5 and 0.36µg/L Alzira and Valencia, respectively), are below the limits HBM-I=0.5 µg/L and HBM-II 2 µg/L established by the HBM Commission. Because the urinary excretion of Cd is proportional to the body burden and therefore represents an indicator of lifetime exposure.

Nickel is a naturally occurring element. The most common harmful health effect of nickel in humans is an allergic reaction. Approximately 10-20% of the population is sensitive to nickel. Significant differences are observed between those children who live near an industrial area (p-value 0.0087). This can be due to the fact that Ni seems to also be associated with the level of urbanization content in automobile exhausts, in bitumen, the main component of asphalt, and in road dust (Alimonti et al., 2000). The geometric mean concentration found (3.90 and 5.83µg/g creatinine as the location studied) is more than double that of other studies such as Heitland & Koster, 2006;). Only the maximum value or levels at 95th percentile reported by Aguilera et al, (2010) and Heitland & Koster (2006) are similar or even higher than our results.

3.3 Relationship between diet and metals

Figure 2 shows the correlations between groups of food and metals. Overall, correlations greater than 0.2 were found to be significant ($\alpha = 5\%$), based on the hypothesis test for the Pearson correlation coefficient, which was computed through the application of SPSS algorithms in our data (Blalock, 1972). According to this criterion, results show that children who ate more vegetables, fruits, cereals and baked products and fish had higher values in metals. Interestingly, for elevated amounts of vegetable consumption, high values of some particular metals were found: Cd, Cu, Mo, Sb, Tl, V, and Zn. Similar results were found by Noli (2016) who pointed out that main elements (Na, K, Fe and Zn) are abundant in all type of vegetables. Zn is abundant in all type of vegetables, but mainly present in cucumber and parsley.

Our results further confirm that fish consumption is linked to the presence of Hg in urine, although methylmercury (MeHg) is the predominant form in fish, and this toxic mercury is mainly detected in blood and hair (Saravanabhavan et al., 2017). In general, the population does not face a significant health problem from methyl mercury, although certain groups with high fish consumption may attain higher risk values. Another important factor related with the increase of Hg in urine is the role of dental amalgams (Health Canada, 2010). The present study has no data available to investigate on the association between both, though this relationship was found by Al-Saleh and Al-Sedairi, (2011).

According to EFSA (2009), the largest contributors to overall total arsenic exposure are fish and cereals. The inorganic arsenic (iAs) is mainly present in cereals, while organic As is present in fish. Our findings show that the frequent consumption of cereals and baked products involves higher values of As in urine. On the contrary, we have not found a correlation between fish consumption and total As levels in urine. Similar results were obtained by Williams et al., (2007) who determined that cereal-based products are the predominant route of exposure to i-As in the EU, with rice as the main contributor. In the Valencian region (Spain), cereals have also been described has the main source for inorganic arsenic exposure through diet (Marin el al. 2017). In general, arsenic usually reaches the human body through plants which grown in soils rich in arsenic or that have been irrigated with contaminated groundwater (Panaullah et al., 2009).

Regarding the rest of elements, correlation with groups of food are not as strong, however other authors relate Cs with vegetables and spices, lunch meat cans and nuts (Health Canada, 2015). Pb, in turns, is related to cereals, pulses, tubers and nuts (Marin et al., 2017).

4. Conclusion

In the present pilot study we have determined the levels of 20 elements in urine of children and studied their relationship with their diet. The results have allowed us to determine the viability of the implemented methodology for developing large biomonitoring studies linked to the food safety risk assessment.

In general, the levels of metals found in the urine of the studied population are similar to the concentrations reported in other studies and are lower than the recommended or health-based guideline value (e.g. As and Hg). On the other hand, no significant differences were found between the levels of metals detected in children living in the rural and urban area, except for Co and Hg.

Children with higher consumption of vegetables, presented significant higher levels of Cd, Cu, Mo, Sb, Tl, V and Zn. Likewise, higher consumption of fish and cereals, were correlated with higher concentration of total Hg and total As, respectively.

For a better interpretation of biomonitoring data including evaluation of highly-exposed population groups, statically-based reference values (RV_{95}) are necessary for the different elements, mainly the toxic metals, in different geographical areas. Likewise, to interpret biomonitoring data in a risk assessment context, more health-based human biomonitoring values are required.

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Study limitations

The participants that joined the programme were recruited from just two locations that covers only a part of the Valencian territory. Likewise, the sample size is reduced and lack of equality between the age/gender. Consequently, the sample is not representative and the conclusions should not be extrapolated to the entire population. Likewise, for some metals, the questionnaire employed does not include questions to identify confounding factors such as the presence of amalgam fillings what can influence the level of total mercury in urine.

Acknowledgments

We would like to thank Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO- Public Health, who supported the research in all its stages, and the children and families from the public schools García Lorca located in Alzira and Padre Català located in Valencia, for their participation and collaboration in this study.

Funding sources

This study was part of the research project CIPAV supported by the Consellería de Educación, Generalitat Valenciana (GV/2011/007).

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