Title: Survey of total mercury and arsenic content in infant cereals marketed in Spain and estimated dietary intake.

Abstract:

Due to the fact that infants and children are especially sensitive to mercury and arsenic exposure, predominantly through diet, a strict control of the most widely consumed infant foods, especially infant cereals, is of paramount importance. Levels of both total mercury and arsenic in 91 different infant cereals from ten different manufacturers in Spain were determined by flow injection adapted to cold vapor and hydride generation atomic absorption spectrometry, respectively. Cereals were assessed in terms of the different types, the predominating cereal in the formulation, the added ingredients, and whether the cereal was organically or conventionally obtained. In general, the content of toxic elements (median (Q_1;Q_3)) found in infant cereals based on conventionally obtained raw materials (n=74, Hg: 2.11 (0.42;4.58), As: 21.0 (9.4;50.9) $\mu g \cdot Kg^{-1}$) was lower than in cereals produced by organic methods (n= 17, Hg: 5.48 (4.54;7.64), As: 96.3 (87.5;152.3) $\mu g \cdot Kg^{-1}$). Mercury content in infant cereals shows the higher values in those formulations with ingredients susceptible to particulate contamination such as gluten-free or cacao-based cereals. The highest arsenic content appears in the rice-based cereals. The mercury and the inorganic arsenic dietary intakes for infants fed on the infant cereals studied were assessed, taking into account the different stages of growth. Organic infant cereals based on cocoa showed the highest risk intakes of mercury, very close to exceeding the intake reference. Just the opposite, 95% of the organically produced infant cereals and 70% of the conventional gluten-free infant cereals showed an inadmissible risk of arsenic intake. Thus, it seems prudent to call for continued efforts in standardizing routine quality control and in reducing arsenic levels in infant cereals; in addition it is essential that relevant legislation be established and regulated by EC regarding these two toxic elements.

Keywords: mercury, arsenic, infant cereals, dietary intake, food analysis, flow injection atomic absorption spectroscopy.

1. Introduction

There are several elements that, far from manifesting deficient pathologies in organisms, they are characterized by demonstrated signs of high toxicity. Two such elements are mercury and arsenic, currently considered to be contaminants according to the food regulations established in several countries (Melo, Gellein, Evje, & Syversen, 2008).

The distribution of mercury throughout the environment is the result of natural and anthropogenic processes involving three main chemical forms: metallic mercury, organic mercury and inorganic mercury compounds, having different characteristics and distinct toxic effects on human health (ATSDR, 2006; Viñas, Pardo-Martínez, López-García, & Hernández-Córdoba, 2001). Arsenic is widely distributed throughout the environment via air, soil, water, and most plant and animal tissues, due to the high mobility of their chemical compounds (Cook, Weinstein, & Centeno, 2005; Mandal, & Suzuki, 2002). This metalloid is present under a number of different chemical inorganic (arsine (AsH_3), trioxide (As_2O_3 or As_4O_6), arsenic trichloride and several arsenates (lead arsenate)) and organic forms (dimethylarsinic acid (DMA) or $(CH_3)_2AsO(OH)$ and arsenobetaine ($CH_3As^+CH_2COO^-$) or (AsBet) monomethylarsenic acid (MMA), trimethylarsine oxide (TMAO), trimethylarsenic (TMAs), arsenocholine (AsC), derived dimethylarsinoribose (arsenosugars) and aryl compounds) involved in complex biological and chemical processes (Guzmán-Mar, Hinojosa-Reyes, Mizanur-Rahman, & Skip-Kingston, 2009; Al Rmalli, Haris, Harrington, & Ayub, 2005; Reilly, 2002).

The accumulation of these elements in the human body is predominantly caused by diet (Martí-Cid, Llobet, Castell, & Domingo, 2008). Mercury intake is primarily through eating fish and marine mammals (Nardi et al., 2009; Qiu et al., 2008) and arsenic usually reaches the human body through plants that have been irrigated with contaminated groundwater (Panaullah et al., 2009; Meharg et al., 2008b). As a result, rice has been shown to be one of the highest arsenic-contributing foods to the human diet (Sun et al., 2009; Meharg, & Jardine, 2003). Another route of exposure for these contaminants is inhalation; in the case of mercury, medical waste incineration or crematoria are involved, and in the case of arsenic, the pathways of exposure in rich environments include contaminated aerosols or agriculture (ATSDR, 2007). In addition, arsenic may reach the human body by dermal and ocular routes as well as through water (Panaullah et al., 2009; Sun et al., 2008).

Mercury is a global pollutant and extremely toxic, especially when metabolized to methylmercury, causing severe symptoms of intoxication and poisoning (Qiu et al., 2008). Several studies suggest that mercury lacks a threshold limit below which no adverse effects occur (ATSDR, 2006). Likewise, inorganic arsenic is classified by the IARC (International Agency for Research on Cancer) as a human carcinogen (group 1) of skin and lungs (Williams et al., 2009; Jorhem et al., 2008). The toxicity of arsenic decreases with increasing methylation (Guzmán-Mar, Hinojosa-Reyes, Mizanur-Rahman, & Skip-Kingston, 2009), but the trivalent forms of MMA and DMA can be carcinogens that are just as potent as inorganic arsenic (Al Rmalli, Haris, Harrington, & Ayub, 2005). The special sensitivity shown by children and pregnant women causes these groups to be severely affected by these toxic elements, exhibiting the same symptoms that occur in adults; negative effects on fetal brain development have also been observed (Drum, 2009).

Typically, plants and vegetables contain low concentration levels because plants absorb only limited amounts of mercury through their roots, even in highly contaminated soils. Approximately 10% of inorganic mercury is absorbed through diet, while the rate of absorption of methylmercury is much higher (95 %) (Nasreddine, & Parent-Massin, 2002; Viñas, Pardo-Martínez, López-García, & Hernández-Córdoba, 2001).

Arsenic is detectable in the majority of foods (Reilly, 2002). The levels of arsenic in fish, shellfish and seaweed are higher than those found in cereals, vegetables and meats, but this element is mostly present in its organic form which has low toxicity (Narukawa, Inagaki, Kuroiwa, & Chiba, 2008; Delgado-Andrade, Navarro, López, & López, 2003). Among cereal products, rice is particularly susceptible to the accumulation of arsenic in comparison to other agricultural products. Inorganic arsenic and DMA are the predominant species found in rice grains (Sun et al., 2009; Zhu, Williams, & Meharg, 2008; Williams, Raab, Feldmann, & Meharg, 2007; Meliker, Franzblau, Slotnick, & Nriagu, 2006; Meacher et al., 2002). Therefore, the cereal products that are based on or mixed with rice represent the principal route of exposure to inorganic arsenic.

The food composition data for these toxic elements, usually obtained from surveillance programs, provides information on total content. From the public health point of view, it allows the levels of these contaminants in commonly consumed foods to be monitored, taking into account the special population groups of risk. In spite of the fact that cereals are not the major contributors to mercury and arsenic intake in the adult diet, the high intake of cereal-based foods by infants and the resulting serious implications regarding

infant health call for a strict control of these elements in infant foods. Consequently, both mercury and arsenic levels found in infant foods are of concern (Meharg et al., 2008c).

At this point, it is appropriate to mention that the food regulations in force in European Countries (Regulation EC 629/2008, EC 1881/2006) (EC, 2008b; 2006a), Commission Directives (2008/60/EC, 2008/84/EC, 2008/128/EC) (EC, 2008a; 2008c; 2008d) and Codex Alimentarius (Codex Stan 193-1995) set maximum permitted limits for total mercury and arsenic in certain specified foods, mainly fish, crustaceans and mollusks but not in other highly consumed foods by special groups of risk such as infants. In this sense, the chemical safety monitoring of the most widely used infant foods, especially infant cereals, is of paramount importance because of the relatively large amount of cereals consumed and the noticeable contribution to dietary intake at an early age.

Nevertheless, the provisional tolerable weekly intake (PTWI) proposed by the Joint Expert Committee on Food Additives (FAO/WHO) made a distinction between total mercury (5.0 $\mu g \cdot Kg^{-1}$ body weight) and its organic form (1.6 $\mu g \cdot Kg^{-1}$ body weight). Subsequently, the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM) established a value of the methylmercury PTWI of 1.6 $\mu g \cdot Kg^{-1}$ body weight (FAO/WHO, 2004) and the U.S. National Research Council (NRC) provided a more restrictive intake limit of 0.7 $\mu g \cdot Kg^{-1}$ body weight per week (EFSA, 2004). In the same way, the FAO/WHO set a PTWI for arsenic of 15 $\mu g \cdot Kg^{-1}$ body weight for inorganic arsenic, considered to be no longer appropriate by the EFSA Panel. A range of values for the 95 % lower confidence limit of the benchmark dose of 1 % extra risk ($BMDL_{01}$) instead of a single reference point has been established, to be applied to the inorganic arsenic risk characterization, 0.3 to 8 $\mu g \cdot Kg^{-1}$ body weight per day (EFSA, 2009; Guzmán-Mar, Hinojosa-Reyes, Mizanur-Rahman, & Skip-Kingston, 2009; Jorhem et al., 2008).

Taking into consideration all these aspects and due to the scarce amount of studies reported in the reference literature (Carbonell-Barrachina et al., 2012), this work has a twofold analytical aim for describing the contents of mercury and arsenic in different types of infant cereals marketed in Spain. The first aim is to assess the major contamination sources of the infant formulations studying i) the repercussion of the cereal production method (organic or conventional), ii) the type of infant cereal, iii) the influence of the predominant cereal in the formulations and, iv) levels provided by different manufacturers, on the final content of these toxic elements; the second aim is

to establish the contribution of analyzed contaminants in the infant diet by means of assessing the level of exposure and potential risk provided by the different analyzed cereals, in order to obtain reliable intake data regarding these toxic elements and infants, and subsequently compare them with the values proposed by the EFSA.

2. Materials and methods

2.1 Infant cereal samples

A sampling of a total of ninety-one infant cereals marketed in Spain from eight different conventional (Hero baby, Milupa, Nestlé, Nutriben, Nutricia, Ordesa, Puleva and Sandoz-Sanutri) and two organic (Biocrecimiento and El Granero Integral) manufacturers was carried out from 2009 to 2010. These samples were provided free of charge by the manufacturers or they were purchased in pharmacies and specialized organic feeding shops from Pamplona (Navarra, Spain). These infant feeding products are usually marketed in a cardboard box of 300 g or 600 g, containing powdered infant cereal packaged in a foil pouch. Infant cereals were preserved in their original package prior to analysis.

The studied samples were grouped according to the mode of cereal production, conventional (n=74) or organic (n=17). In addition, the current market offers a wide variety of infant cereal formulations which can be classified by considering two basic aspects: the recommended growth stage and the different main ingredients that enrich the cereal formulation (cocoa, fruits, honey, milk, multicereals and gluten-free). In a chronological order of supply, infant formula or human milk are initially supplemented with gluten-free (gluten-free-based infant cereals, n=30, a specialized formula which is normally based on rice and corn) and/or fruit cereals (infant cereals with fruits, n=7, a formula composed with- or without-gluten-based cereals and added dehydrated fruits) especially designed for infants from 4 to 6 months of age. Subsequently, gluten multicereals (n=25, an infant product based on mixed gluten cereals formulated for satisfying the needs of infants from 5 to 12 months old) enriched with milk (n=5, a formula containing follow-up infant formula which constitutes the principal liquid source of nourishment for infants as of six months of age), honey (n=17, a formula similar to multicereal with the addition of honey, but especially designed for infants from 6 months to 2 years of age) and cacao (n=7, a formulation which is normally recommended for infants as of 12 months of age) are incorporated into the infant

feeding regimen. The cereals included in the multicereal formulation are rice, corn, wheat, oats, rye, millet, sorghum and barley.

According to the predominant cereal used in the different formulations, infant cereals were classified as follow: wheat (n=5), oats (n=4), mixed cereals (combinations of five (n=12) or eight (n=35) different cereals), rice (n=13), corn (n=1) and mixture of rice and corn (n=21).

2.2 Chemicals and Reagents

The highest purity reagents available were used throughout the study. All materials were washed with a solution of 2 % Triton X-100 (Sigma, Barcelona, Spain), soaked in 10 % (v/v) nitric acid for at least 3 days and then carefully rinsed with ultrapure water before use.

The standard and sample solutions were prepared using Milli-Q ultrapure deionized water. The mercury and arsenic stock standard solutions (1000 $mg \cdot L^{-1}$) were purchased from Scharlau Chemie and Merck (Barcelona, Spain), respectively. Sub-boiled nitric acid (obtained from 65 % nitric acid analytical grade, Merck), which was additionally purified by sub-boiling distillation in a quartz sub-boiling still (Hans Kürner, Rossenheim, Germany), was added to the working standard solutions to match the acid concentration of digestion solutions.

Hydrogen peroxide (30 % w/v, pro analysis Panreac, Barcelona, Spain), hydrochloric acid (37 %, pro analysis Panreac), ascorbic acid (Analar Normapur, VWR Prolabo, Barcelona, Spain) and potassium iodide (pro analysis Panreac) were used for treating the samples.

Sodium borohydride (pro analysis Merck) and sodium hydroxide (pro analysis Panreac) were used as a reducer and hydrochloric acid (pro analysis Panreac) was used as the carrier.

Finally, NCS ZC 73008 Rice and NCS ZC 73009 Wheat (China National Analysis Center for Iron and Steel, Beijing, China) certified reference materials were used in the optimization and validation of the methodology.

2.3 Instrumental

For the digestion process of the infant cereal samples, a Milestone Ethos Plus microwave with a high pressure segmented rotor (HPR-1000/10S) and temperature sensor (Milestone, Sorisole, Italy) was used.

The analytical determination of mercury and arsenic was carried out using a Perkin Elmer AAnalyst 800 atomic absorption spectrometer (Norwalk, CT, USA) equipped with a flow injection valve (FIAS 100), an AS-90 auto-sampler and deuterium background correction. Single mercury and arsenic electrodeless discharge lamps providing resonance lines of 253.7 and 193.7 were operated at 180 and 380 mA, respectively and with a slit width set at 0.7 nm. Integrated absorbance peak areas were measured using a quartz cell supplied by Perkin Elmer.

The temperature of the quartz cell was 100 ºC for mercury and 900 ºC for arsenic. Hydrochloric acid was used as the carrier at concentrations of 3 % and 10 % for mercury and arsenic, respectively. A mixed solution of 0.2 % sodium borohydride in 0.05 % sodium hydroxide was used as the reducer.

2.4 Analytical procedure

The following items were placed inside each high-pressure Teflon vessel: 0.5 g of infant cereal, 7 mL of sub-boiled nitric acid and 2 mL of hydrogen peroxide. These digestion bombs suffered an optimized temperature profile program in the microwave digestion system (Stage 1: 25-140 ºC, for 5.5 minutes, Stage 2: 140-150 ºC for 4 minutes, Stage 3: 150 ºC for 7 minutes; Stage 4: 150-180 ºC for 10 min, Stage 5: 180 ºC for 20 minutes; all steps at 1000 W, immediately followed by ventilation at room temperature for 15 minutes).

The acid digested sample solution was diluted in a 10 mL volumetric flask with ultrapure water and then transferred to previously acid-cleaned and labeled polypropylene tubes. The tubes were then stored in refrigeration at – 20 ºC. Three replicated digestions were made for each sample. Details of measurements and analytical procedures are discussed in other publications (Hernández-Martínez, & Navarro-Blasco, 2012; Sola-Larrañaga, & Navarro-Blasco, 2009). Before the analytical determination of mercury was carried out, each digestion sample underwent argon bubbling for 4 minutes in order to remove the NOx dissolved gases. An additional preparation of the sample is required in order to assure the reduction of total arsenic. Therefore, 5 mL of digested solution were added to a beaker, and placed on a hotplate until total dryness. Then 1 mL of 50 % solution hydrochloric acid and 1 mL of a solution containing 5 % potassium iodide and ascorbic acid were added to the residue. It took 45 minutes to carry out reduction of the total arsenic. After this time had elapsed, the final solution was diluted in a 10 mL volumetric flask with ultrapure water,

transferred to acid-cleaned labeled polypropylene tubes and stored frozen at -20 ºC until analysis.

2.5 Quality assurance

Reliability of both mercury and arsenic analytical methodologies was assured by means of two certified reference materials NCS ZC 73008 Rice and 73009 Wheat, under specified conditions. In-house quality control (Hg: 0.504 ± 0.009 $\mu g \cdot L^{-1}$, range: 0.492-0.539, n=36; As: 3.03 ± 0.09 $\mu g \cdot L^{-1}$, range: 2.87 - 3.16, n=36) and blank reagent were also tested in order to satisfy the criteria established in the quality program (lower and upper action limits, Hg: 0.450 and 0.550 $\mu g \cdot L^{-1}$, As: 2.7 and 3.3 $\mu g \cdot L^{-1}$, respectively) and to provide on-going quality control information throughout the course of the study. The analytical blank data was taken into account when correcting the concentration values corresponding to the studied samples. The relative standard deviation of the method was determined in the intra-assay precision (aliquots of samples measured during the same assay session) for the internal aqueous control of mercury and arsenic (1.1 and 2.9 %, respectively). As well the values obtained for inter-assay precision (aliquots of the same sample measured on different days) were 3.1 and 3.2 %, respectively.

The accuracy of the method was demonstrated by the agreement found in the obtained results (Hg: 5.59 ± 0.01 and 1.28 ± 0.09 $ng \cdot g^{-1}$, As: 0.094 ± 0.009 and 0.027 ± 0.002 $\mu g \cdot g^{-1}$, respectively) with the 95 % confidence interval certified values (Hg: 5.3 ± 0.5 and 1.6 $ng \cdot g^{-1}$, As: 0.102 ± 0.008 and 0.031 ± 0.005 $\mu g \cdot g^{-1}$, respectively). In addition, recoveries of the analyzed elements, carried out by spiking the NCS ZC 73008 rice samples before digestion, were satisfactory, 105.2 ± 3.7 % and 109.7 ± 4.2 %, respectively.

Furthermore, standard addition technique was performed on reference material for evaluating the presence of matrix interferences. A comparison of slopes using external standard calibrations (Hg: $y=0.0084x + 0.0001$, $r^2=0.9998$ and As: $y=0.0350x + 0.0136$, $r^2=0.9973$) versus standard additions in NCS ZC 73008 Rice (Hg: $y=0.0090x + 0.0042$, $r^2=0.9992$ and As: $y=0.0379x + 0.1737$, $r^2=0.9967$) showed good agreement. The comparison of the confidence intervals of the slopes (Hg: 0.0084 ± 0.0004 vs. 0.0090 ± 0.0011; As: 0.035 ± 0.004 vs. 0.038 ± 0.006) by means of *t*-student test reveals statistical support to this finding, indicating that the matrix has no effect on the analysis of mercury or arsenic.

In accordance with the definition and criteria established by IUPAC ($x_b + 3\sigma_b$), the limit of detection (LOD) was calculated as the average of three times the standard deviation of the reagent blank, setting at 0.014 $\mu g \cdot L^{-1}$ for mercury and 0.31 $\mu g \cdot L^{-1}$ for arsenic (n=12), equivalent to 0.27 and 2.30 $\mu g \cdot Kg^{-1}$ respectively, when expressed in terms of infant cereal.

2.6 Statistical analysis

SPSS (Statistical Package for the Social Sciences) program version 15.0.1. was used in this study for all the statistical analyses of the data. Initially, the Kolmogorov-Smirnov statistic was used to determine whether or not the data followed a normal distribution. All data were taken into account for the statistical study. Samples below the LOD were assumed to be at a concentration value half that of the LOD. Due to the lack of normality, the different groups of infant cereal samples (classified according to cereal production methods, type of cereal, predominant cereal and infant cereal manufacturers) were compared via a non-parametric Kruskal-Wallis test and a Mann-Whitney U-test with a statistical significance set at $p<0.05$.

3. Results and discussion

Frequency analysis of mercury and arsenic concentrations in the studied infant cereals shows a positive skew, where the majority of the data of both distributions are clustered to the left at low values. In addition, the distributions exhibit positive kurtosis values, indicating a rather peaked leptokurtic distribution for arsenic and, to a lesser extent, for mercury. The result of the normality test and the inspection of the normal probability plot (Normal Q–Q plot) suggest a violation of the assumption of normality. It allows the use of median (the most representative parameter) and interquartile range (Q_1;Q_3) as helpful information regarding the statistical description of data. Mean and standard deviation are also provided as additional informative values.

Mercury concentration resulted in ranges between <0.27 and 13.96 $\mu g \cdot Kg^{-1}$, which has a median (lower and upper interquartile) of 2.61 (0.66;5.13) and a mean of 3.51 $\mu g \cdot Kg^{-1}$, respectively. Arsenic shows a median (Q_1;Q_3) of 24.2 (11.0;84.5) $\mu g \cdot Kg^{-1}$, and a mean of 51.0 $\mu g \cdot Kg^{-1}$ ranged between 2.4 and 346.3 $\mu g \cdot Kg^{-1}$.

The variability in the content of the studied elements may be due to several factors. Primarily, different cereals (wheat, oats, barley, rye, sorghum, millet, rice, maize),

predominant ingredients, and other major components of the formulation (milk, fruits, cocoa, honey) are the main sources of these metallic contaminants (Francesconi, 2007).
In addition, potential mercury and arsenic contamination of the added mineral salts and trace elements, additives allowed in processed cereal-based foods by Directive 2006/125/EC (EC, 2006b), provides a secondary source of these potentially toxic compounds. Both contributions are evident in EFSA and the scientific cooperation SCOOP 3.2.11 studies on contaminants in the food chain carried out for assessing the extent of dietary exposure from different food groups and periods of age (EFSA, 2009; 2004). These aforementioned studies were prior to the establishment of Regulation 1881/2006 (SCOOP, 2004).
In the reference literature, there is very little information regarding the contents of arsenic and mercury in the food matrix analyzed in this study. Therefore, a qualitative comparative study was carried out using just the cereal items previously published in total diet studies (Table 1). In spite of the analytical difficulties encountered in the quantitative determination of mercury and the low concentration found, it is possible to establish a similarity in the findings. Most of the mercury concentration values found in the item cereals were generally included in the analytical range of determination (<0.27-13.96 $\mu g \cdot Kg^{-1}$), with the exception of the high levels provided by certain cereal samples from Germany, Portugal and France. The analyzed concentration range of total arsenic includes almost all of the targeted values shown in Table 1, close to the levels found in Germany and Chile, and significantly higher than those provided by Canada and the United Kingdom samples.

3.1 Influence of cereal production method on mercury and arsenic contents in infant cereals

In this study, lower toxic element values are apparent in infant cereals based on raw materials obtained in a conventional way (Hg: 2.11 (0.42;4.58), As: 21.0 (9.4;50.9) $\mu g \cdot Kg^{-1}$) compared to organically produced infant cereals (Hg: 5.48 (4.54;7.64), As: 96.3 (87.5;152.3) $\mu g \cdot Kg^{-1}$). This fact was confirmed by high statistical significance when compared by means of a Mann-Whitney U-test ($p<0.001$). The same behavior is observed in previous studies on lead, cadmium and aflatoxins in the infant cereals (Hernández-Martínez, & Navarro-Blasco, 2012; 2010). On the contrary, other reported studies suggest little difference in the arsenic content found in organic vegetables with respect to their ordinary or conventional production (Ghidini et al., 2005); only

selective, occasional and sporadic contaminations may explain high levels of these contaminants (Malmauret, Parent-Massin, Hardy, & Verger, 2002).

The natural conclusion of these results leads to the assertion that the contents of these potentially toxic elements are affected by environmental conditions of the system, for example wet or dry industrial pollution and the rate of rainfall in the crop development season; in addition, the impact on soil after pH modifications or bioaccumulation of metals, resulting from the application of fertilizers over several decades, may determine the metal contamination for life regardless of the culture system used, be it organic or conventional. The legacy of the prolonged use of phosphorus fertilizers contaminated with arsenic drastically represents a clear example of this fact (Malmauret, Parent-Massin, Hardy, & Verger, 2002; Scoullos, Vonkeman, & Makuch, 2001; Jorhem, & Slanina, 2000).

3.2 Mercury and arsenic content in the different types of infant cereal

Table 2 shows the descriptive statistical parameters and concentration distributions of the analyzed contaminants according to the different types of marketed cereals for infants; in turn, these data are distinguished between organic and conventional cereals.

The concentration range of total mercury in infant cereals samples is virtually unchanging, as expected in this type of food. It is noteworthy to point out the larger amount of contamination found in formulations with ingredients which are more susceptible to a particulate contamination, such as cocoa (organic: 10.10; conventional: 3.60 (0.74;4.86) $\mu g \cdot Kg^{-1}$), gluten-free (organic: 5.48 (4.01;7.27) $\mu g \cdot Kg^{-1}$·, conventional: 3.99 (1.00;4.72) $\mu g \cdot Kg^{-1}$) or cereal with fruits (organic: 7.41 (5.11;9.71) $\mu g \cdot Kg^{-1}$·, conventional: 2.08 (0.82;2.15) $\mu g \cdot Kg^{-1}$), than in the cereals with honey (organic: nd; conventional: 0.40 (<0.27;3.29) $\mu g \cdot Kg^{-1}$) or in the cereals with milk (conventional: 0.50 (0.36;1.30) $\mu g \cdot Kg^{-1}$), as shown by the low contents in both types, conventional and organic, provided by the literature (Lee et al., 2006; Devillers et al., 2002).

The highest total concentration of arsenic is provided by the gluten-free cereals (organic: 152.3 (92.3;204.8) $\mu g \cdot Kg^{-1}$, conventional: 77.4 (51.7;98.0) $\mu g \cdot Kg^{-1}$) in relation to gluten infant cereals (i.e., multicereals, organic: 100.3 (93.8;115.7) $\mu g \cdot Kg^{-1}$, conventional: 21.6 (9.7;24.4) $\mu g \cdot Kg^{-1}$) that exhibit notable homogeneity (Table 2). This finding was easily explained, mainly due to the fact that the former group uses rice as the main component (Sun et al., 2009; Meliker, Franzblau, Slotnick, & Nriagu, 2006). At the same time, the fact that the rice grain provides a relatively high percentage of

toxic arsenic (approximately 30-95 %) is of great concern (Díaz et al., 2004; Muñoz et al., 2002).

3.3 Mercury and arsenic concentrations in different types of cereals with regard to the predominating cereal

Vast food technology permits the preparation of various formulations adapted to the infant nutritional requirements in cereals at every stage of their growth. Currently, there is a very broad range of infant cereals on the market and the innovation being carried out is constantly increasing with the introduction of functional ingredients for different purposes. However, the predominant cereal in different types of infant cereals has been maintained over recent decades and can be any one of the following: wheat, oats, mixed cereals (combinations of five or eight different cereals), rice, corn, or a mixture of rice and corn. In order to identify the enrichment sources of the analyzed contaminants, it is desirable to classify the infant cereals according to the main cereal used in the formulation (Table 3).

The total mercury contents in organic and in conventional formulations differ markedly from each other in the diverse matrices studied, with the exception of the rice-based preparations (organic: 4.54 (4.01;6.52) $\mu g \cdot Kg^{-1}$, conventional: 4.39 (0.74;7.51) $\mu g \cdot Kg^{-1}$), whose total mercury contents represent the minimum and maximum, respectively, of all the other infant cereals studied.

However, within the same cereal production system, the mercury contents in the different formulations (shown in table 3) are relatively homogeneous, and even the extreme values did not exceed the levels normally found in the published literature (ranges found in rice: 1.6-353, oats: 9-20, barley: 3.4-19, rye: 3-18, wheat: 0.9-21 $\mu g \cdot Kg^{-1}$ (Qian et al., 2010; Nardi et al., 2009; Qiu et al., 2008; Food Standard Agency, 2006; Rivero-Huguet, Huertas, Francini, Vila, & Darré, 2006; Al-Saleh, & Shinwari, 2001; Kabata-Pendias, 2001).).

Table 3 shows the highest level of total arsenic found in rice-based formulations (organic: 154.9 (126.0;204.7), conventional: 96.3 (71.7;99.7) $\mu g \cdot Kg^{-1}$) and in mixed rice and corn (organic 88.2 (84.5;152.3) and conventional: 60.5 (43.7;82.4) $\mu g \cdot Kg^{-1}$) which coincide with the high value observed in the rice plantations (Spain: 105 ± 4 $\mu g \cdot Kg^{-1}$ (Matos-Reyes, Cervera, Campos, & de la Guardia, 2010); United Kingdom: 183 $\mu g \cdot Kg^{-1}$ (Food Standard Agency, 2006); 57-318 $\mu g \cdot Kg^{-1}$ (Meharg et al., 2008c); Sweden: 40-63 $\mu g \cdot Kg^{-1}$ (Jorhem et al., 2008); United States of America: 30-110 $\mu g \cdot Kg^{-1}$ (Tao, &

Bolger, 1998); 170 $\mu g \cdot Kg^{-1}$ (Williams, Raab, Feldmann, & Meharg, 2007); Japan: 120-280 $\mu g \cdot Kg^{-1}$ (Sun et al., 2009); China: 8-490 $\mu g \cdot Kg^{-1}$ (Qian et al., 2010); Canada, France, and Venezuela: 8-202 $\mu g \cdot Kg^{-1}$ (Zavala, & Duxbury, 2008)).

In addition, Du, Zhu, Liu, & Zhao (2005) describe a negative interaction between the total mercury and arsenic content in the rice plant. This finding has been proven and can be strongly verified in the organic rice-based preparations, (As: 154.9 (126.0;204.7), Hg: 4.54 (4.01;6.52) $\mu g \cdot Kg^{-1}$) and, to a lesser degree, in the preparation based on a mixture of rice and corn (As: 88.2 (84.5;152.3), Hg: 5.76 (5.11;9.71) $\mu g \cdot Kg^{-1}$).

Moreover, the tendency to use integral rice in organic formulations may, in itself, represent an enrichment of arsenic because this metalloid is, for the most part, located on the surface of the grain in the pericarp and in the aleurone (Meharg et al., 2008c).

In the rest of the formulations based on gluten cereals, the level of concentration is almost constant (organic: oats, 90.5; five-mixed, 92.7; eight-mixed, 100.3; conventional: wheat, 15.6; oats , 12.3; five-mixed, 13.1; eight-mixed, 12.0 $\mu g \cdot Kg^{-1}$) and fortunately, significantly lower with respect to the previously mentioned infant cereals.

In brief, with regard to total mercury and arsenic levels, the rice-based infant cereals need a strict control on the part of the manufacturer due to the fact that the levels found are very high and because these formulations are given to infants in the first stage of neonate life.

3.4 Manufacturers and the levels of mercury and arsenic in infant cereal

Figure 1 shows the box plot diagrams of both distributions of data according to commercial infant cereal manufacturers. It should be noted that the analyzed infant cereals belong to a total of ten different manufactures, eight of which correspond to conventional production (companies 1-8) and the other two corresponding to organic production (trading houses 9-10); these 10 companies supply the majority of the infant cereal sold in Spain.

The organic companies 9 and 10 were notable for their low industrial processing control measures regarding infant cereal formulations and their arsenic levels (97.0 (74.9;179.5) and 95.1 (88.9;152.3) $\mu g \cdot Kg^{-1}$), as well as their mercury levels (5.15 (4.01;8.68) and 5.58 (5.11;7.39) $\mu g \cdot Kg^{-1}$). Within the group of conventional manufacturers, 7 and 6 were the companies that, from an analytical perspective, exerted the least production control regarding arsenic and mercury, respectively (Figure 1). The rest of the infant cereal manufacturers performed proper food control of mercury and arsenic

contaminants in the infant cereal formulations. It should be pointed out that, from a global point of view, the commercial houses 2 and 3 showed excellent quality control, with a wide range of different formulations included on their product list (12 and 19 infant cereals, respectively).

In short, the conventional manufacturers work with more stringent food safety criteria than the organic companies. An improved and comprehensive examination of raw materials and food processing is essential in order prevent the appearance of these elements in the final product or at least to keep them in check.

3.5 Estimated dietary intake of mercury and arsenic

Infant cereal is the traditional choice of the first solid food for infants. It is often mixed with infant formula or breast milk to give a slurry consistency and constitute a basic integral part of an infant diet during the first stages of life. Therefore, infant cereals constitute a potential vehicle for toxic elements and they are the main source of intake of these contaminants. The estimation of theoretical dietary intake of mercury and arsenic via the studied cereals is of particular significance.

Dietary intake was calculated using the mercury and arsenic median content determined in the different types of infant cereal according to feeding tables and recommended doses, suggested by manufacturers, for the various stages of infancy (4 months: 48 g; 5 months: 60 g; 6 months: 72 g; 7-12 months: 84 g; 13-24 months: 96 g) and mean body weight (4 months: 6.5 kg; 5 months: 7.25 kg; 6 months: 7.75 kg; 7-12 months: 9 kg; 13-24 months: 13 kg).

Figure 2 shows the estimated dietary intake of total mercury and inorganic arsenic for infants fed on the studied infant cereals in comparison with the more restrictive tolerable weekly intake (TWI) value for methylmercury recommended by NRC and the inorganic arsenic reference range set by CONTAM Panel, taking into account the different growth stages (EFSA, 2009; 2004).

The studies on dietary intake of mercury provided by infant cereal in the infant diet are scarce and most of them are based on total dietary mercury intake (Wilhelm, Wittsiepe, Schrey, Lajoie-Junge, & Busch, 2003; Noble, & Emmett, 2001). The comparison of dietary intake with the tolerable intake of organic mercury is complex. However, the reference value of the TWI established by NRC can be interpreted as a dose that is safe to take on a weekly basis. Taking this fact into consideration, the conventionally produced infant cereals contributed the lowest daily mercury intake, from 0.03 to 0.35

$\mu g \cdot day^{-1}$; most of the organic cereals gave an intermediate intake, from 0.10 to 0.53 $\mu g \cdot day^{-1}$; and the cocoa-based organic cereals provided the highest intake (0.85-0.97 $\mu g \cdot day^{-1}$). These findings coincide with the values provided by the group of cereals in the total intake studies (0.05- <1.5 $\mu g \cdot day^{-1}$, Rubio, Gutiérrez, Burgos, & Hardisson, 2008; Muñoz et al., 2005; Llobet, Falcó, Casas, Teixidó, & Domingo, 2003; Larsen et al., 2002; Cuadrado, Kumpulainen, & Moreiras, 1995), pointing to a single source of pollution: the anthropogenic industrial activity (Nasreddine, & Parent-Massin, 2002). As can be observed in figure 2, none of the studied infant cereals exceeded the mercury TWI. Organically produced cereals ranged between 0.011 and 0.094 $\mu g \cdot day^{-1}$ Kg^{-1} (10.8-94.4 % of TWI), with the cereals containing cocoa reaching the maximum value; and conventional cereals remained below 0.033 $\mu g \cdot day^{-1}$ Kg^{-1} (33.5 % of TWI), in accordance with the established PTWI percentages for total diet studies (1.6-36 %, Lee et al., 2006; Nasreddine et al., 2006; Larsen et al., 2002; Ysart et al., 1999; Urieta, Jalon, & Eguileor, 1996; Becker, & Kumpulainen, 1991) which considered that the largest source of mercury contamination lies in fish and shellfish.

Total arsenic is usually analyzed for estimating dietary exposure in total diet studies, although the major signs of toxicity lie essentially in the inorganic form. Based on preliminary studies (Carbonell-Barrachina et al., 2012; Sun et al., 2009; Meharg et al., 2008a,c), the estimated percentage of inorganic arsenic in cereals with gluten reaches almost 100 % (98 ± 1 %) while the value for rice-based infant cereals is lower (64 ± 5 %). Based on this assumption, after an easy transformation according to the ratio total-inorganic arsenic content, an appropriate final estimation of arsenic risk exposure is obtained (Lee et al., 2006).

The organic infant cereals supplied a dietary intake (multicereals: 0.81-0.92; cereal with cocoa: 0.66-0.84; gluten-free cereals: 0.72-0.81; cereal with fruits: 0.63-0.79; cereal with honey: 0.37-0.46 $\mu g \cdot day^{-1}$ Kg^{-1}) that is included in the range of the benchmark dose level ($BMDL_{01}$) set for inorganic arsenic, and which involves an inadmissible risk for infant health. It is important to point out the high contribution intake of conventional rice-based cereals (0.37-0.41 $\mu g \cdot day^{-1}$ Kg^{-1}) which might be considered a health hazard for infants. Yet the rest of the conventional cereals, providing the lowest inorganic arsenic intake (multicereals: 0.18-0.20; cereal with cocoa: 0.13-0.16; cereal with fruits: 0.14-0.18; cereal with milk: 0.07-0.09; cereal with honey: 0.06-0.08 $\mu g \cdot day^{-1}$ Kg^{-1}), do not appear to be a health risk.

This higher intake of gluten-free formulations is consistent with arsenic intake values provided by the group of cereals (1.3-18.2 $\mu g \cdot day^{-1}$, Jorhem et al., 2008; Martí-Cid, Llobet, Castell, & Domingo, 2008; Bocio, Nadal, & Domingo, 2005; Muñoz et al., 2005; Delgado-Andrade, Navarro, López, & López, 2003; Llobet, Falcó, Casas, Teixidó, & Domingo, 2003), mainly rice, reflecting their greater environmental burden and their impact on the general population.

Finally, the dietary intake reference values made it possible to calculate, taking into account the aforementioned feeding tables and recommended dose, the upper content of both mercury and arsenic, which could provide an infant cereal with no risk of adverse effects in toddlers and infants. The estimated values reached an upper limit of 10.71-13.54 $\mu g \cdot Kg^{-1}$ for mercury and a range of 56.6-1692.7 $\mu g \cdot Kg^{-1}$ for arsenic. Only two cereal formulations (conventional multicereal, 13.22 $\mu g \cdot Kg^{-1}$ and organic gluten-free cereal, 13.96 $\mu g \cdot Kg^{-1}$) exceeded the calculated maximum mercury level and could be considered under suspicion. A great number of infant cereals (32 out of 91 cereals) provided a high arsenic level, including almost all the organically produced infant cereals (16 out of a total of 17 studied formulations) and 70 % (16 out of 23 cereals) of the conventional gluten-free infant cereals. Therefore, these findings are worthy of consideration due to the possibility that early-life exposure might lead to impaired infant development. In this respect, cereal products intended for infants require special attention and a more exhaustive control. Hence, it seems reasonable to ask that more efforts be made to control the raw materials used in the manufacturing process of infant cereals and to demand an EC regulation for infant cereals, especially in the formulations with more potential risk impact, such as the gluten-free-based cereals.

4. Conclusion

Infant cereals must not jeopardize infant health with excessive exposure to mercury or arsenic. It has been shown that infant cereals based on raw materials obtained in a conventional way contribute significantly lower amounts of both of the studied toxic elements than those based on raw materials obtained in an organic way. Therefore, manufacturers of infant formulations based on organic cereals should routinely monitor their products and carry out a more rigorous control so as to keep toxic elements at an absolute minimum.

Although the studied infant cereals varied little in mercury content, with increased content in those formulations containing ingredients (cocoa, gluten-free cereal and fruit)

that are more susceptible to particulate air contamination, the highest values of arsenic found in gluten-free rice-based formulations are of concern. The exposure of arsenic from infant foods must be taken into serious consideration, principally when the rice used has been grown in arsenic-rich soil, probably treated with arsenical pesticides of unstated composition. It is also important when brown rice has been used in the manufacturing of infant cereals because inorganic arsenic is mainly located in the bran layer of unpolished rice. Likewise, based on these findings and on the present knowledge regarding mercury and arsenic toxicity, and taking into account the non-existent European Community regulation regarding this matter, it is essential to ask that a maximum guideline value be established for total mercury as well as for total arsenic, including an additional safety factor for infant cereals with values close to the aforementioned upper limits, 10 and 56 $\mu g \cdot Kg^{-1}$, respectively.

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Table 1. Mercury and arsenic concentrations (μg·Kg^{-1}) of the group of cereals expressed by total diet studies.

Country (Region)	Reference	Hg	As
Spain (Andalucía)	Cuadrado et al., 1995	2.0	143 (<LOD-444)
Spain (Cataluña)	Martí-Cid et al., 2008	<0.1*	45
Spain (Canarias)	Rubio et al., 2008	0.4	-
Spain (Madrid)	Cuadrado et al., 1995	4.5	-
Spain (Galicia)	Cuadrado et al., 1995	0.4	-
Spain (País Vasco)	Urieta et al., 1996	<5	<5
Spain (Valencia)	Cuadrado et al., 1995	0.8	-
Spain (This study)	-	2.61(0.66-5.13)	24.2(11.0-84.5)
China	Chung et al., 2008	<LOD	-
Chile	Muñoz et al., 2005	8	25
Canada	Dabeka et al., 1987	-	8.6
France	SCOOP, 2004	19(3-115)	<12.5
Germany	SCOOP, 2004	7(<1-490)	50(<10-300)
Portugal	SCOOP, 2004	56(30-90)	<100
United Kingdom	SCOOP, 2004	4(<1-9)	7(3-21)

*ng·Kg^{-1}

Table 2. Mercury and arsenic content in different types of infant cereal (µg Kg^{-1}).

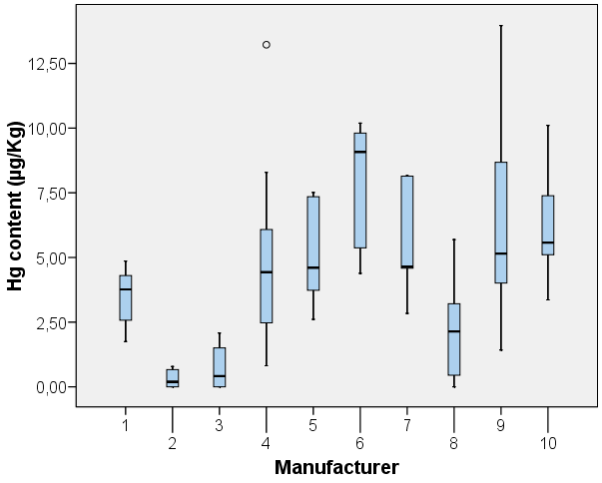
Analyte	Type	Production	n	Mean	Std. dev.	Median	(Q_1;Q_3)
	Cocoa	Conventional	6	3.10	2.17	3.60	(0.74;4.86)
		Organic	1	10.10	-	-	-
	Fruits	Conventional	5	1.81	1.52	2.08	(0.82;2.15)
		Organic	2	7.41	3.26	7.41	(5.11;9.71)
	Honey	Conventional	16	2.04	2.99	0.40	(<LOD;3.29)
		Organic	1	1.42	-	-	-
Hg	Milk	Conventional	5	0.96	1.05	0.50	(0.36;1.30)
		Organic	-	-	-	-	-
	Multicereal	Conventional	19	3.61	3.89	2.15	(0.67;5.96)
		Organic	6	5.90	1.34	5.51	(5.15;7.39)
	Gluten-free	Conventional	23	3.43	2.64	3.99	(1.00;4.72)
		Organic	7	6.48	3.74	5.48	(4.01;7.27)
	Cocoa	Conventional	6	17.6	6.0	18.0 [a,b]	(11.8;23.4)
		Organic	1	91.7	-	-	-
	Fruits	Conventional	5	23.7	21.9	19.7 [a,b]	(9.1;24.0)
		Organic	2	86.7	3.2	86.7	(84.5;88.9)
	Honey	Conventional	16	11.7	8.0	8.2 [a]	(5.0;17.8)
		Organic	1	50.8	-	-	-
As	Milk	Conventional	5	10.7	3.5	10.3 [a,b]	(8.7;13.7)
		Organic	-	-	-	-	-
	Multicereal	Conventional	19	17.7	8.8	21.6 [b]	(9.7;24.4)
		Organic	6	107.2	34.0	100.3	(93.8;115.7)
	Gluten-free	Conventional	23	74.9	31.6	77.4 [c]	(51.7;98.0)
		Organic	7	165.2	101.7	152.3	(92.3;204.8)

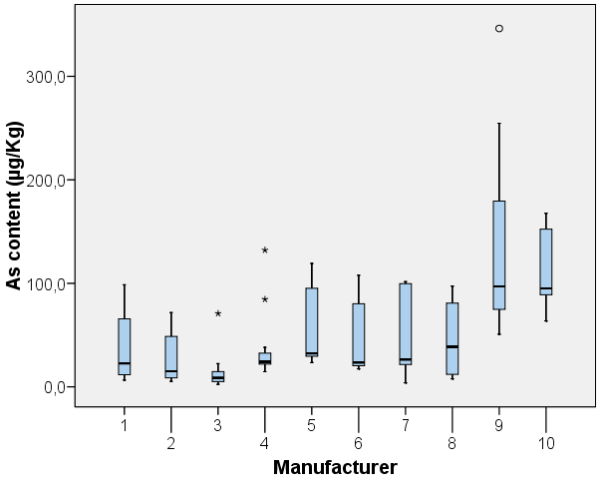
[a, b, c] Homogenous subsets from Mann-Whitney U-test

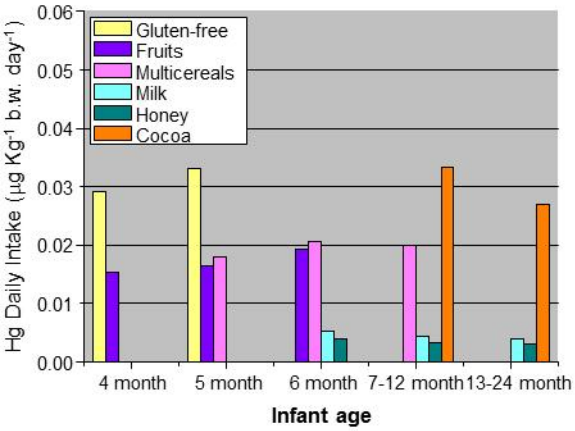
Table 3. Mercury and arsenic content in infant cereal according to the predominant cereal used in the infant formulation (μg Kg^{-1}).

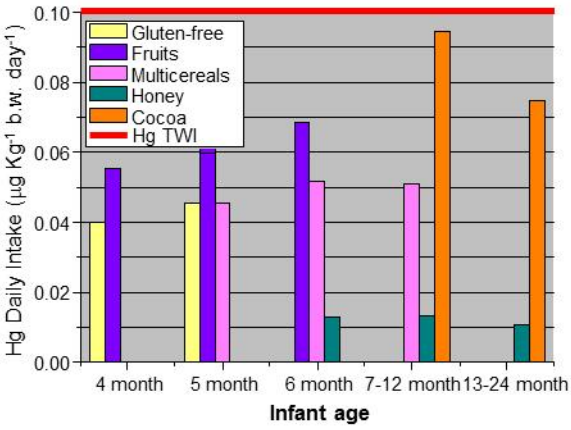
Analyte	Type	Production	n	Mean	Std. dev.	Median	(Q_1;Q_3)
Hg	Wheat	Conventional	5	1.83	2.22	0.74	(0.42;2.61)
		Organic	-	-	-	-	-
	Oats	Conventional	2	0.36	0.52	0.36	(<LOD;0.73)
		Organic	2	6.66	1.40	6.66	(5.67;7.64)
	Mixed 5	Conventional	10	3.39	4.21	2.25	(<LOD;4.86)
		Organic	2	7.17	4.16	7.17	(4.23;10.10)
	Mixed 8	Conventional	31	2.72	3.08	1.75	(<LOD;3.88)
		Organic	4	4.83	2.48	5.25	(3.29;6.36)
	Rice	Conventional	10	4.17	3.33	4.39	(0.79;7.51)
		Organic	3	5.51	2.65	4.54	(4.01;6.52)
	Rice+Corn	Conventional	15	2.80	1.75	2.42	(1.61;4.41)
		Organic	6	7.28	3.88	5.76	(5.11;9.71)
	Corn	Conventional	1	0.80	-	0.80	-
		Organic	-	-	-	-	-
As	Wheat	Conventional	5	15.0	7.4	15.6 [a]	(10.1;20.4)
		Organic	-	-	-	-	-
	Oats	Conventional	2	12.3	4.0	12.3 [a]	(9.4;15.1)
		Organic	2	90.5	35.6	90.5	(65.3;115.7)
	Mixed 5	Conventional	10	16.7	8.1	13.1 [a]	(10.2;22.7)
		Organic	2	92.7	1.5	92.7	(91.7;93.8)
	Mixed 8	Conventional	31	14.2	8.8	12.0 [a]	(6.4;22.6)
		Organic	4	104.8	48.1	100.3	(73.6;135.9)
	Rice	Conventional	10	89.9	23.4	96.3 [b]	(71.7;99.7)
		Organic	3	168.9	79.8	154.9	(126.0;204.7)
	Rice+Corn	Conventional	15	60.9	31.3	60.5 [c]	(43.7;82.4)
		Organic	6	137.2	106.7	88.2	(84.5;152.3)
	Corn	Conventional	1	3.1	-	3.1 [a,b,c]	-
		Organic	-	-	-	-	-

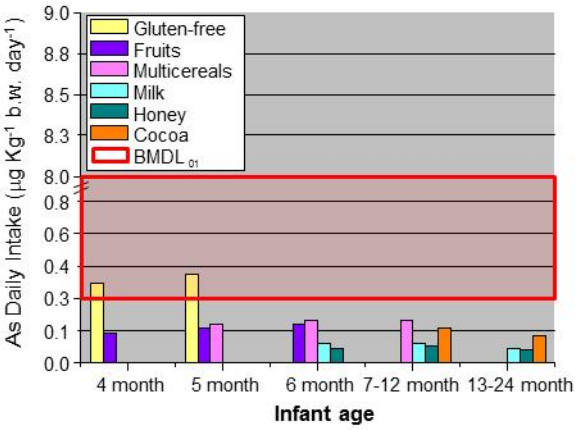
[a, b, c] Homogenous subsets from Mann-Whitney U-test


Hg content (µg/Kg)
12,50
10,00
7,50
5,00
2,50
0,00
1
2
3
4
5
6
7
8
9
10
Manufacturer


As content (µg/Kg)
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8
9
10
Manufacturer


Gluten-free
Fruits
Multicereals
Milk
Honey
Cocoa
Hg Daily Intake (µg Kg-1 b.w. day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
4 month
5 month
6 month
7-12 month
13-24 month
Infant age


Gluten-free
Fruits
Multicereals
Honey
Cocoa
Hg TWI
Hg Daily Intake (µg Kg-1 b.w. day-1)
0.10
0.08
0.06
0.04
0.02
0.00
4 month
5 month
6 month
7-12 month
13-24 month
Infant age


Gluten-free
Fruits
Multicereals
Milk
Honey
Cocoa
BMDL 01
As Daily Intake (µg Kg-1 b.w. day-1)
9.0
8.8
8.5
8.3
8.0
0.8
0.6
0.4
0.3
0.1
0.0
4 month
5 month
6 month
7-12 month
13-24 month
Infant age

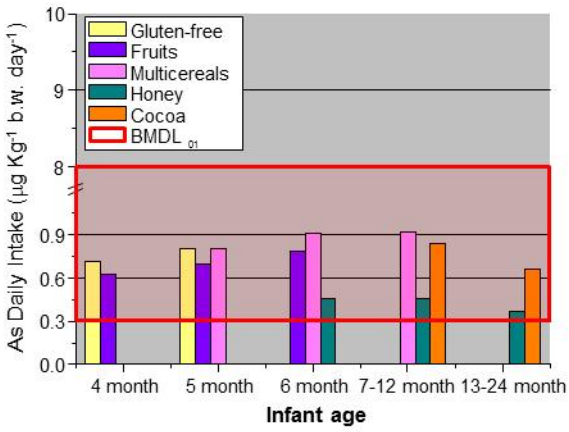

Gluten-free
Fruits
Multicereals
Honey
Cocoa
BMDL 01
As Daily Intake (µg Kg-1 b.w. day-1)
10
9
8
0.9
0.6
0.3
0.0
4 month
5 month
6 month
7-12 month
13-24 month
Infant age

Figure 1. Box plot of mercury and arsenic distributions in infant cereals provided by different conventional (1-8) and organic (9-10) manufacturers ($\mu g \cdot Kg^{-1}$).

Figure 2. Total mercury and inorganic arsenic estimated dietary intake for infants fed on the studied infant cereals.