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Selenium and cadmium in bioaccessible fraction of organic weaning food: Risk assessment and influence of dietary components

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ABSTRACT

Background: The tendency of some sectors of the population to consume organic food has also come to include baby food. Nevertheless, it is necessary to develop studies to support the true nutritional and toxicological value of these products, making special emphasis in several trace elements. To our knowledge, no studies have been conducted on this type of organic food.

Methods: Weaning foods with different formulations categorized as organic were analyzed to determine Se and Cd contents as well as its bioaccesibility. The analyses were conducted by electro thermal atomic absorption spectroscopy (ET – AAS) after the treatment of the samples with acid mineralization. Besides, macronutrient analyses (protein, fat and dietary fiber) were also developed. Finally, a novelty statistic approach such as @Risk was used to evaluate contributions to DRI or PTWI of Se and Cd derived for consumption of these weaning foods.

Results: Se content ranged between 2.44–15.4 μg Kg^{1}. Samples with meat ingredients showed the highest Se contents, while weaning foods consisting of fruits or vegetables presented the lowest concentrations. Se bioccessible concentration ranged between 1.90–4.35 μg Kg^{-1} with a greater uniformity amongst analyzed samples. Regarding Cd, concentrations of this heavy metal ranged between 1.23 and 3.64 μg Kg^{-1}. Furthermore, Cd bioaccessibility of organic weaning foods ranged between 0.17 and 1.38 μg Kg^{-1}. The solubility of all samples studied was around 20% from the initial Cd concentration. A negative statistical correlation between fat content – Cd bioaccesible ($p < 0.05$; $r = -0.756$) and Cd content – Se bioaccesible ($p < 0.05$; $r = -0.777$) were also found.

Conclusions: Cd concentrations are considerably lower than those reported in weaning formulas which were not categorized as organic. On the other hand, the analysed organic jars did not represent a significant source of Se. The probabilistic assessment developed, showed that contributions to DRI of Se for infants 1–3 years old by consumption of these weaning foods, are excessively low (15% at best).

1. Introduction

Currently, organic food production is one of the fastest growing markets, with an increase of around 250% in the last 10 years [1]. The principal reason is that organic agriculture can enhance human and environmental health because there is no use of synthetic fertilizers or pesticides. Nevertheless, the lack of confidence in the labeling of many organic products, the reduced half-life of these foods compared to conventional ones and higher production cost, make it is necessary to develop studies to support the true nutritional and toxicological value of these products [1]. Research data on the nutritional benefits of organic food compared to their non-organic homologues is scarce and contradictory [2,3].

The tendency of some sectors of the population to consume organic food has also come to include baby food. However, the nutritional composition of this baby food should be monitored very strictly as these infant foods must provide high biological value proteins, energy and micronutrients. One of these micronutrients considered essential to the human body is selenium (Se). This trace element plays an important role in several physiological functions, such as forming part of enzymes like glutathione peroxidase [4], thioredoxin reductase [5] and iodothyronine-5 deiodinase [6]. A low maternal Se status during pregnancy has been related to fetal malformations, like neural tube defects [7], and is disadvantageous for cognitive development in infants and

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Table 1
Ingredients used in the formulation of weaning food analyzed (data supplied by manufacturer).

	Code	Ingredients
Banana, Pear and Pomegrate	BPP	Fruit 97.5% (banana, pear, pomegranate juice, lemon juice), flour 2.5%, vitamin C.
Vegetables, Chickpeas and Pear	VCP	Cooking water and vegetables 45.5% (potato, squash, tomato, onion, green beans), chickpeas 6%, pear 3%, flour 2.5%, extra virgin olive oil 2%, paprika spices0.14%.
Vegetables, Veal	VV	Vegetables 67.5% (tomato, potato, onion, peas, carrot), veal 12%, cooking water, flour 2.5%, extra virgin olive oil and spices 2%.
Banana, Apple, Orange	BAO	Fruit 100% (banana, apple, orange juice, lemon juice), vitamin C.
Multifruit	MFR	Fruit 95.5% (banana, apple, orange juice, for, grape juice, lemon juice), carrot juice 4.5%, vitamin C.
Zucchini Creme	ZC	Vegetables 75% (zucchini, potato, leek, onions), cooking water, extra virgin olive oil.
Squash, Chamomole Flower	SCF	Cooking water and vegetables 69.5% (squash, potato, onion, leek), extra virgin olive oil 2%, peppermint and chamomile flower.
Varied Vegetables Creme	VVC	Cooking water and vegetables 72% (potato, leek, green beans, onion, zucchini) and extra virgin olive oil 2%.
Vegetables, Chicken	VC	Cooking water and vegetables 56% (potato, tomato, onion, red pepper), chicken 12%, extra virgin olive oil 2% parsley and spices.
Vegetables, Chicken, Pasta	PVC	Cooking water and vegetables 51% (potato, tomato, onion), chicken 12%, wholemeal pasta 4%, extra virgin olive oil 2% and spices.

toddlers [8,9]. However, Se may also be toxic for many organisms when it is presented in high concentrations, being one of the chemical elements in which there is less difference between essential and toxic levels [10].

On the other hand, cadmium (Cd) is a ubiquitous heavy metal, widespread in agriculture as a consequence of the use of pesticides. It is expected that organic agricultural practices result in a lower content of Cd in food produces. In an infant´s early life, low-level Cd exposure through breast milk could induce oxidative stress [11]. Besides, Cd could be stored in different organs (lungs, kidney, digestive system, bone tissue, gonads) and remains in a child's body until adulthood [12] (up to 30 years). In addition, it has also been identified as a potent neurotoxin [13]. Finally, data from animal experiments show that the gastrointestinal absorption of Cd in newborns is significantly higher than in adults [14].

In order to evaluate the nutritional and toxicological value of food, it is necessary not only to determine the trace element or heavy metal concentration initially present but also evaluate its bioaccessibility. This term refers to the amount of the inorganic element (trace element or heavy metal) that is found soluble in the intestinal lumen after an *in vitro* simulated digestion; therefore, this element is available to be absorbed by the enterocytes. This bioaccessibility is influenced by the presence of other components present in the food matrix (fiber fractions, peptides from protein digestion, vitamins, etc.). Therefore, it is of foremost relevance when developing food formulations to know how food components influence bioaccessibility by increasing or decreasing trace element absorption.

Several studies have been made concerning Se and Cd bioaccessibility in food matrices such as pulses [15], cereals [16], and fish [17] in the case of Se, and vegetables [18], shellfish [19,20], and pulses [15] with Cd. However, as far as we know, only one study was found which assessed bioaccessibility of Cd in weaning foods, nevertheless its ingredients were not labeled as organic [14]. Given the above, the objective of this article was i) to determine the bioaccessibility and the total content of Se and Cd present in weaning food categorized with the "organic" attribute ii) to study the influence of other food components such as protein, fiber and fat in the bioaccessibility of these elements; iii) to develop a probabilistic/risk assessment study of Se and Cd intake by consumption of these organic weaning foods with a novel approach (@Risk) in order to evaluate the nutritional/toxicological value of these products.

2. Materials and methods

2.1. Materials and reagents

All reagents were of analytical-reagent grade. Ultrapure water (18 MΩ/SCF) prepared with a Milli-Q Reference Water Purification (Millipore, Madrid, Spain) was used throughout experiments. All glassware and plastic containers were soaked in 10% nitric acid overnight and rinsed three times with de-ionized water prior to use. Nitric acid (65%), hydrochloric acid (35%) and sulphuric acid (97%) were obtained from Panreac (Barcelona, Spain). Sodium bicarbonate (97%) and sodium hydroxide were obtained from Scharlab (Barcelona, Spain). Magnesium nitrate hexahydrate (98%) and magnesium oxide (98%) were obtained from Alfa Aesar (Kandel, Germany).

Digestive enzymes and bile salts were supplied by Sigma-Aldrich Co. (St. Louis, MO). The working solutions of these enzymes were prepared immediately before use. Pepsin solution was obtained by dissolving 3.2 g of pepsin (P-7000 from porcine gastric mucosa) in 20 ml of HCl (0.1 M). The solution of pancreatin and bile salts were prepared by dissolving 0.6 g of pancreatin (P-3292 from porcine pancreas) and 3.9 g of bile salts (B-8756 of porcine origin) in 150 ml of 0.1 M $NaHCO_3$.

Enzymes used for fibre assays (α-amylase heat stable; protease from *Bacillus licheniformis*; amyloglucosidase from *Aspergillus niger*) were obtained from Sigma-Aldrich Co. (St. Louis, MO). Standard solutions for measuring the elements Se and Cd were prepared immediately before use by dilution with distilled deionised water of 1000 mg/L standard solutions (Scharlau Chemie, Barcelona, Spain).

2.2. Samples

Se and Cd contents were analyzed in 10 commercialized weaning foods characterized with the “organic” attribute (jars of 200 g). These weaning foods were received at the University of Cordoba (Spain) proceeding from a factory specialized on the manufacture and marketing of organic food. Upon arrival at the laboratory, samples were poured in plates, freezed – dried and packed in polypropylene vacuum bags, until required for analyses.

All ingredients used in the formulation of different weaning food are described in Table 1. For mineral content determination, a total of 50 samples were analyzed, corresponding to one sample taken from five different jars for each type of weaning food. In order to get a representative sample, this procedure was repeated for the bioaccesibility assay. The total samples analyzed in both determinations were 100.

2.3. Procedure for in vitro gastrointestinal digestion

The bioaccessible fraction of weaning foods, was obtained through an *in vitro* process of gastrointestinal digestion simulated based on the one described by Cámara et al. [21]. The procedure consisted on measuring 3 g of lyophilized sample of weaning food and adjusting pH to 2.0 using 6 N HCl. The first stage required a pepsin – HCl digestion in which 0.5 g of pepsin solution for each 100 g of homogenized was added (corresponding to 0.125 g of porcine pepsin by 3 g of lyophilized sample). The mixture was then incubated for 2 h at 37 °C in a shaking water bath (HSB-2000 Shaking Bath; E-Chrom Tech CO., LTD, Taipei, Taiwan). After this time, to stop gastric digestion, the sample was maintained for 10 min in an ice bath.

Following, the pH was adjusted to 5 by adding 1 M $NaHCO_3$ to continue with intestinal digestion step. Then, 6.2 mL of a mixture of pancreatin and bile salts (corresponding to 0.025 g of pancreatin and

0.160 g of bile salts by 3 g of lyophilized sample) was added to each test tube, which was incubated for 2 h more. After Intestinal digestion, flasks with samples were submerged for 10 min in an ice bath to stop the action of this mixture of enzymes.

Finally the pH was adjusted to 7.2 with 0.5 M NaOH. Aliquots of the digested sample were transferred to polypropylene centrifuge tubes (50 ml, Costar Corning Europe, Badhoevedorp, The Netherlands) and these were centrifuged for 1 h at 4000 rpm and 4 °C. (eppendorf Centrifuge 5810 R) Then, the supernatant (soluble fraction) was collected, its organic matter was destroyed, and the mineral content was measured by atomic absorption spectrometry.

2.4. Trace element determination

To determine Se and Cd content of weaning foods 1 g of lyophilized sample was weighed in a porcelain crucible. Samples were treated with 1.5 mL of ashing aid suspension (20% w/v $MgNO_3$ and 2% w/v MgO) and 5 mL of 7 M HNO_3 in order to avoid Se volatilization. The mixture was evaporated on a heating plate at 80 ºC until total dryness. Subsequently, samples were incinerated in a muffle furnace at 460 ºC for 15 h. The ash was bleached after cooling by adding 2.5 mL of 2 N HNO_3, drying on thermostatic hotplates, and maintaining in a muffle furnace at 460 °C for 1 h more. Ash recovery was performed with 1 mL of HCl 6 N, making up to 10 mL with deionised water. Se and Cd content present in bioaccessible fraction obtained in the previous process were determined in a similar way.

A Perkin-Elmer model AAnalyst 600 atomic absorption spectrometer equipped with a Perkin-Elmer model FIAS 400 hydride generation system and an autosampler was used for Se measurements. Analytical determinations of Cd were measured by graphite furnace atomic absorption spectrometry in a Perkin Elmer model AAnalyst 600 equipped with a Zeeman furnace module and an autosampler AS-800, controlled by the WinLab 32 software. Electrodeless discharge lamps, operated from an external power supply were used. The instrumental parameters are derived from previous optimization works [17,22] (Tables 2 and 3). The accuracy and precision of the different analytical techniques used while determining Se and Cd concentrations were validated by recovery experiments using CRMs (Table 4).

AOAC methods (2005) were used to determine the proximate composition: defatting in a Soxhlet apparatus with petroleum ether for crude fat (920.85) and micro-Kjeldahl for protein (920.87). Briefly, to Kjeldahl method 0.5 g of lyophilized sample with a catalyst pellet was placed in digestion flask and 20 mL of concentrated H_2SO_4 were added. The mixture was heated in a digester until solution clears. After cooling with 70 mL of deionised water, the solution was distilled with a small quantity of NaOH, which converts the ammonium salt to ammonia. The ammonia gas is led into a trapping solution of HCl 0.1 N and determined by back titration with NaOH solution 0.1 N. The protein concentration was calculated from the nitrogen values using a conversion factor of 6.25. For the determination of fat content, 3–5 g of lyophilized sample were weighed and wrapped in a paper filter, and subsequently transferred into a Soxhlet liquid/solid extractor with petroleum ether during 1 h. After fat extraction, samples were dried in desiccators and weighed.

Dietary fibre (total, soluble and insoluble) were determined by the enzymatic gravimetric method of the AOAC (991.43) [23]. The basis of this method is the isolation of dietary fiber from the rest of components by enzymatic digestion (Step 1: amylase – T = 95°C – t = 30 min; Step 2: protease – T = 60°C – t = 30 min; Step 3: amyloglucosidase – T = 60°C – t = 30 min). After all these steps, ethanol is added to precipitate the soluble fibre (this step is avoided in the insoluble fiber determination). Finally, the residue is filtered in crucibles with 0.5 g of celite, washed with 78% and 95% ethanol and acetone and dried to measure the weight of residue.

Table 2
Instrumental conditions for determination of Cd (λ = 228.8 nm) by ET – AAS.

Step	Temperature (ºC)	Ramp (s)	Hold (s)	Argon flow (mL/min)
1	130	10	45	300
2	300	10	20	300
3	800	10	20	300
4	1450	0	4	0
5	2600	2	2	300

ET – AAS: Electrothermal Atomic Absoprtion Spectroscopy.

Table 3
Instrumental conditions for determination of Se (λ = 196 nm) by HG – ET - AAS.

Step	Temperature (ºC)	Ramp (s)	Hold (s)	Argon Flow (ml/min)
1	250	1	50	0
2	250	1	20	250
3	1950	0	5	0
4	2300	1	3	250

Step	Time (s)	Speed of pump 1 (rpm)	Speed of pump 2 (rpm)
Prefill	15	100	0
Fill	15	100	0
Fill	5	100	80
Injection	30	0	80

FIAS flow injection program used for the hydride generation-AAS measurements.
Prefill step: FIAS autosampler sampling tube rinsed with simple solution (only done for the first replicate).
HG – ET –AAS: Hydride Generation with Electrothermal Atomic Absorption Spectroscopy.

2.5. Statistics and risk assessment

The data were analyzed using SPSS 15.0 (IBM, Armonk, NY). In order to validate the normality of the data obtained, the Shapiro-Wilks test was used. Later, Pearson's correlation (parametric conditions) was used for determining the dependence between variables. Significant differences were considered when $p < 0.05$.

A probabilistic model was developed to estimate the intake level for Se and Cd derived from consumption of weaning foods. The model here developed followed a probabilistic approach in which variables were described by probability distributions. They were fitted to concentration data obtained in our study for each element (total element concentration and bioaccessible element concentration). Furthermore, in order to estimate the intake level, serving size was considered. However, since no data were found concerning the consumption patterns of this type of product, we assumed a serving size ranging from 150 to 200 g for infants (one jar) between 1 and 3 years old, which was defined by a uniform distribution in the probabilistic model; meaning that all values in that range had the same probability to occur.

The probability distributions describing the Se and Cd concentration data were fitted by using @Risk v7.5 (Palisade, Newfield, NY, USA). The simulation was run using 100,000 iterations for each element. The goodness of fit to data was assessed by using different statistical tests which corresponded to Akaike Information Criterion (AIC) test and Chi-square test. These statistical tests allow researchers to give a guess of how well the fitted distribution described the observed data. In addition, the visual analysis was equally considered to assess the fit of the probability distributions to intake data.

3. Results and discussion

3.1. Selenium

Se content in organic weaning foods ranged between 2.5–15.4 μg Kg^{-1} (Table 5). In accordance with previous studies that show protein

Table 4
Analysis of certified reference materials for Se and Cd.

CRM	Se (mg/Kg)			Cd (mg/Kg)		
	Found	Certified	Recovery (%)	Found	Certified	Recovery (%)
Spinach leaves (SRM – 1570)	0.111 ± 0.007	0.115 ± 0.004	95			
Bovine liver (BCR – 185R)	1.64 ± 0.04	1.68 ± 0.14	98	0.530 ± 0.003	0.544 ± 0.017	97

Table 5
Content of Se and Cd (fresh matter) in the organic weaning food studied (mean ± standard deviation).

Sample	Se total (μg/Kg)	Se soluble (μg/Kg)	Cd total (μg/Kg)	Cd soluble (μg/Kg)
ZC	2.47 ± 0.27	2.04 ± 0.22	1.23 ± 0.75	0.17 ± 0.11
VVC	2.56 ± 0.43	1.96 ± 0.13	1.27 ± 0.34	0.18 ± 0.06
SCF	2.44 ± 0.22	2.91 ± 0.46	2.20 ± 0.32	0.22 ± 0.08
BAO	5.26 ± 0.81	3.97 ± 1.90	2.65 ± 0.23	0.45 ± 0.09
BPP	3.31 ± 1.59	3.41 ± 1.22	2.31 ± 0.57	1.23 ± 0.60
MFR	2.46 ± 0.62	2.44 ± 0.47	2.46 ± 0.74	1.38 ± 0.48
PVC	15.4 ± 1.4	3.76 ± 0.27	3.04 ± 0.50	0.28 ± 0.16
VC	12.8 ± 1.9	1.90 ± 0.81	1.56 ± 0.06	0.22 ± 0.08
VCP	3.70 ± 1.48	3.25 ± 0.24	3.64 ± 0.49	0.23 ± 0.12
VV	8.40 ± 1.11	4.35 ± 1.35	1.59 ± 1.43	0.24 ± 0.06

Table 6
Content of fiber (total and soluble), protein and fat (fresh matter) in the organic weaning food studied (mean ± standard deviation).

Sample	Total fiber (g /100 g)	Soluble fiber (g /100 g)	Protein (g /100 g)	Fat (g /100 g)
ZC	0.90 ± 0.03	0.58 ± 0.04	0.34 ± 0.01	2.46 ± 0.13
VVC	0.67 ± 0.05	0.44 ± 0.03	1.40 ± 0.04	3.12 ± 0.80
SCF	1.60 ± 0.47	1.32 ± 0.16	1.13 ± 0.53	2.76 ± 0.33
BAO	0.47 ± 0.06	0.30 ± 0.14	0.95 ± 0.06	0.65 ± 0.19
BPP	0.92 ± 0.08	0.69 ± 0.17	0.35 ± 0.07	1.02 ± 0.10
MFR	1.13 ± 0.05	0.66 ± 0.05	0.34 ± 0.05	0.87 ± 0.13
PVC	0.75 ± 0.07	0.38 ± 0.09	3.39 ± 0.24	2.36 ± 0.14
VC	0.78 ± 0.02	0.69 ± 0.05	3.24 ± 0.30	3.11 ± 0.25
VCP	2.11 ± 0.02	1.30 ± 0.09	2.75 ± 0.22	1.98 ± 0.19
VV	0.87 ± 0.10	0.58 ± 0.03	2.37 ± 0.17	2.47 ± 0.15

foods are the main dietary sources of Se [24], samples with meat ingredients such as PVC (15.4 μg Kg^{-1}), VC (12.8 μg Kg^{-1}) and VV (8.4 μg Kg^{-1}) showed the highest Se contents, while weaning foods consisting of fruits or vegetables presented the lowest concentrations (SCF, ZC and MFR: 2.5 μg Kg^{-1}). In fact, a positive significant correlation ($p < 0.01$; r = 0.817) was observed between Se content (μg · Kg^{-1}) and protein content (mg 100 g^{-1}). From the data, it is apparent that vegetable based weaning foods generally had a poorer quality in relation to Se than meat based. On the other hand, the results found for all recipes are higher than those reported by Zand et al. [25], who showed values below the limit of quantification (< 2.4 μg Kg^{-1}) for eight brands of infant complementary food from the United Kingdom, in which some of the ingredients used were categorized as organic. Similar values have been reported by Ruiz de Cenzano et al. [26] for commercial baby foods with purees based on fruits and vegetables (5.4–15.4 μg Kg^{-1}) (these ones were not categorized as organic). Besides, these authors show that when other ingredients such as turkey or chicken (21.1–24.6 μg Kg^{-1}) or even fish (sole, sea bass or monkfish) (43.9–109 μg Kg^{-1}) [26] are included in the recipes, Se concentrations are higher. As a result of the scarce literature on Se concentration in baby foods, no conclusions can be drawn about whether the low Se contents found in our baby foods is due to organic ingredients or recipe composition (with little presence of animal – derived ingredients).

Regarding the bioaccessible concentration of Se, a greater uniformity of values was found, which ranged between 1.9 and 4.3 μg Kg^{-1} for VC and VV, respectively (Table 5). Khanam and Platel [16] have reported better Se bioaccessible results for cereal based composite meals (with many ingredients used in our recipes) which ranged between 27–33 μg Kg^{-1}. Bioaccessibility of Se from the organic weaning foods does not seem to be dependent on their total Se content. In fact, samples with the highest Se content containing meat ingredients such as PVC (24%), VC (15%), and VV (52%), presented lower bioaccessibility percentages than those with moderate Se content such as ZC (83%), SCF (100%), BPP (100%) and MFR (99%). These results are in agreement with a similar study in which bioaccessibility of Se was independent of the total content of this trace element in cereals, pulses and leafy green vegetables, making the information on Se bioaccessibility from foods relevant [27].

Despite the strong correlation between protein and Se contents, no significant correlation was found between Se solubility and protein content. Thus, the influence of protein sources over Se bioaccessibility is not clear. Some authors suggest that Se bioavailability decreases when the protein content in samples increases [28]. It seems that amino acids obtained from protein digestion increase the ionic strength of the aqueous phase on intestinal lumen where solubility of Se species decreases. On the contrary, Daniels [29] suggests that Se may be better absorbed from a high protein diet and Yan et al. [30] have reported a linear or log-linear dose-dependent increase between several Se – status indicators and dietary supplementation with protein isolates.

Fat content in weaning foods studied ranged between 0.65 and 3.12 g 100g^{-1} (Table 6). It has been indicated in a previous study that fat content of foods may impair Se bioaccessibility [17] mainly because Se species are poorly lipophilic molecules [28], and fat micelles formed during the digestion process may interfere with enzymes capacity to release Se present in peptide samples of low molecular weight [17]. Although, a negative significant correlation between both nutrients could not be established in the present study, ($p = 0.065$; r = − 0.636), a decrease in Se bioaccessible concentration could be observed in samples with the highest fat content such as VC or VVC.

On the other hand, total dietary fiber contents ranged between 0.47 and 2.11 g 100g^{-1}. These contents are in agreement with previous studies [31], where around 65% corresponded to the insoluble fraction and the remaining 35% to the soluble fraction. Although dietary fiber has traditionally had a negative role on minerals and trace elements´ bioaccessibility, currently there is little objective evidence that dietary fiber *per se* does not have this adverse effect on mineral metabolism [32]. According to our data, no statistically significant correlation was found between total fiber content and Se bioaccessibility in the samples studied. Indeed, some of the samples with the highest total fiber content such as VCP or SCF also presented moderately high amounts of bioaccessible Se (see Tables 5 and 6). Similarly, Baye et al. [33] found through *in vitro* studies that binding properties of some soluble fiber fractions such as gums or pectins are minimal; and even Sakai et al. [34], reported enhancing properties upon trace element absorption for fructo-oligosaccharides, inulin and pectin in rats. In the present study, a neutral effect of fiber could be observed upon Se bioaccessibility, both for soluble fiber and for the insoluble fiber. Therefore, a negative role on mineral absorption of weaning foods cannot be attributed to this nutritional component.

3.2. Cadmium

A recent report commissioned by the European Parliament points out that organic food production restricts the use of pesticides and antibiotics in farmed animals and results in lower concentrations of crop Cd [35]. Exposure to these compounds during pregnancy is associated with negative effects on intelligence and neuro-behavioral development. The greatest hazard of Cd in infancy is related to its capacity to affect the developing nervous system and bone formation [12,36]. In the organic weaning foods studied, Cd content ranged between 1.23 μg Kg^{-1} for ZC and 3.64 μg Kg^{-1} for VCP (see Table 5). These concentrations are considerably lower than those reported in Swedish weaning formulas (1.10–23.5 μg Kg^{-1}) [14] and Pakistani weaning foods (72.2 μg Kg^{-1}) [37], which were not categorized as organic. In fact, Cd content in our organic weaning foods may be considered similar to those reported in some studies with breast milk (mean levels around 2 μg L^{-1}) [38,39]. Nevertheless, these Cd levels in breast mik can be even lower (0.13 μg Kg^{-1}) when the study is performed amongst non – smoking women belonging to rural areas of Bangladesh, with a minimum industrial and traffic pollution [11]. As far as we know, there are few studies conducted to evaluate bioaccessibility and total Cd content in weaning foods and at the moment it is difficult to establish strong conclusions, but it seems using organic ingredients in the formulation of weaning foods could lead to reduced concentrations of this heavy metal.

Cd bioaccessibility of organic weaning foods studied ranged between 0.17 μg Kg^{-1} for ZC and 1.38 μg Kg^{-1} for MFR (Table 5). Throughout all the samples studied solubility was around 20% from the initial Cd concentration. As has already been mentioned, studies focusing on Cd bioaccessibility of infant food are scarce. Nevertheless, these values are lower than those of cooked food matrix such as vegetables (34–64%) [18], green beans (66%), carrots (73%), leek (76%) [40] and rice (70–74%) [41]. Many of these aforementioned ingredients are commonly used in weaning food recipes. Thus, these results, in addition to the initially smaller Cd concentration found in the analyzed samples, could show the benefit of using ingredients classified as organic to make up these kinds of foods.

Protein and dietary fiber contents did not have any statistically significant influence upon Cd bioaccessibility. Nevertheless, a negative statistical correlation was found between fat content and Cd bioaccesible concentration ($p < 0.05$; r = − 0.756) for all samples studied. As has been previously mentioned regarding Se, this interaction can be justified by explaining that fat decreases the rate of enzymatic digestion, which also decreases Cd release from the molecules to which it is bound, given this, its bioaccessibility is also diminished.

Similarly, a negative statistical correlation was found ($p < 0.05$; r = − 0.777) between Cd total content and Se bioaccessible concentration in all the studied samples. Respectively, a similar negative interaction was found between Se total content and Cd bioaccessible concentration; however, in this case it was not statistically significant (p = 0.471; r = − 0.277). These results strengthen the protective role of Se upon heavy metals toxicity such as Cd. Interaction Se – Cd has been previously described in literature with animal models [42,43]. A study conducted with broilers have shown that diets with 0.3 mg Kg^{-1} of organic Se (mainly selenomethionine as Se –yeast) can help against the negative effects of moderate Cd levels in these diets (10 mg Kg^{-1}), but cannot counteract the negative effects of higher doses (100 mg Kg^{-1}) [44]. Likewise, Marval – León et al. [17] have also reported a significant negative correlation between Se bioaccessible and Cd content in several fish species. Similarly, the addition of organic Se in increasing concentrations (from 0.15 to 3 mg Kg^{-1}) could reduce the tissue deposition of Cd concentrations because the bioavailability of both elements decreases [45]. The mechanism which could explain this negative interaction between both elements consists of the formation of complexes or salts, which are either insoluble or poorly soluble in water. However, this will also depend on the chemical form in which Se is present, as well as the Se/Cd ratio [17]. Thus, in opposition to the protective effect of selenomethione discussed above, others previous studies with inorganic Se have shown that 0.1 mg Kg^{-1} Se as sodium selenite, added to diets contained 200 mg Kg^{-1} of Cd as cadmium chloride did not induce any significant changes in the levels of Cd accumulation in rats' kidney, liver [46]. However, another study [47] showed that sodium selenite supplementation in equimolar doses with cadmium chloride (8 μmol during 5–9 days) can decrease Cd retention in the tissues of suckling rats (mainly liver and kidney).

3.3. Probabilistic assessment

As already mentioned in Materials and methods section, a probabilistic model approach was developed to estimate the intake level of Se and Cd, which derived from consumption of 150–200 g (one jar) of these organic weaning foods. Dietary reference intakes (DRI) for the Spanish population of 1–3 years old were considered for Se (20 μg/day) [48]. In the case of Cd, considering that it is a heavy metal, provisional tolerable weekly intake (PTWI) of 2.5 μg/kg body weight · week was used [49]. The models were developed from values of total and bioaccessible (soluble) trace element content. It should also be noted that the present statistical tool was completed using the variability of inorganic elements present in food as well as the variability of the organic weaning foods ingested. Both aspects determine the total amount of inorganic elements ingested.

Gamma distribution showed the best fit to total concentration intakes of Se (AIC = 19609.14; Chi-Square = 60.00) (Fig. 1a). The mean Se intake estimated based on the aforementioned consumption pattern was of 0.96 μg with values of 0.66 μg and 2.91 for 50th and 95th percentiles, respectively. Thus, results derived from the simulation of the probabilistic model indicated that the intake level of Se through the consumption of one jar of these organic weaning foods would be below 2.91 μg for the 95% of infant population (15% of DRI for Se). There would even be individuals who do not exceed an intake above 0.03 μg (5th percentile) of Se from the consumption of these ecological weaning foods. Regarding the bioaccessible Se concentration data, a log normal distribution presented the best fit (AIC = - 1685.18; Chi-Square = 74.36) (Fig. 1b). Estimated intakes were not above 0.52 μg and 1.00 μg for the 50th and 95th percentiles, respectively. In this later case, contributions to DRI of Se for the infant population group would not exceed 5%.

Considering the importance of Se and its important role in antioxidant selenoproteins for protection against oxidative stress, it can be said that the analysed organic jars did not represent a significant source for this inorganic micronutrient, making the search for alternative dietary sources necessary or even the formulation of these weaning foods with improved recipes with a high proportion of animal ingredients (meat or fish).

In the case of Cd, being a heavy metal element, the main objective is to know if the PWTI would be exceeded through the intake of these weaning foods (25–40 μg/week). This PWTI value range was obtained from multiplying 2.5 μg/kg body weight · week by the average body weight relevant for Spanish infants (10, 13 and 16 Kg for toddlers of 1, 2 and 3 years old respectively). The results derived from the simulation of the probabilistic models indicated that the exposure levels of Cd were low, being below the established PTWI. A BetaGeneral distribution was fitted to Cd total intake concentration data (AIC = −5771.05; Chi-Square = 274.91) (Fig. 2a). The mean Cd intake estimated was 0.34 μg and it did not exceed 0.69 μg for the 95th percentile of the studied population. This means that in the worst-case scenario, infants consuming a single serving of these weaning foods (equivalent to one jar) would only reach between 2.7 – 1.7% of PTWI for Cd. Even the maximum value of this distribution would be 0.79 μg (3.1 – 2.0% of PTWI). A log normal distribution was fitted to the bioaccessible Cd concentration intake data (AIC = −37455.58, Chi-Square = 72.34). In this case, the intakes of bioaccessible Cd per jar of ecological weaning

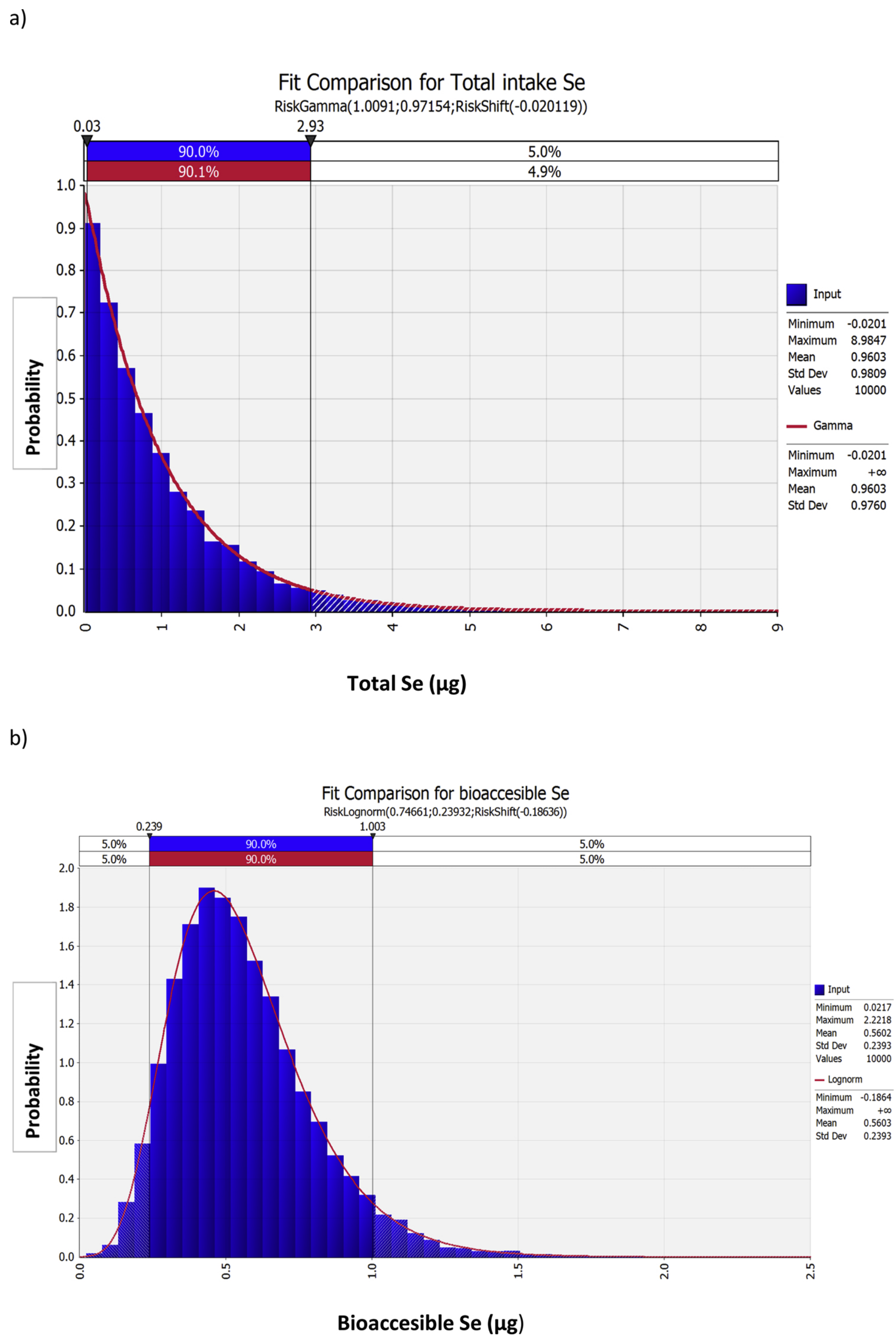

Fig. 1. Simulated data and fitted probabilistic distribution for Se total trace and bioaccessible.

foods would not exceed 0.22 µg for the 95th percentile (about 0.88–0.55% of PTWI).

Although there are some studies in the literature which have determined the concentration of Cd present in infant formulas [50,14] and human milk [51,38], few studies have been conducted to assess the risk of Cd exposure in infants through the consumption of weaning foods. There is just one study conducted by Eklund and Oskarsson [14] in infant formulas and weaning foods reporting an average total Cd intake of 0.44 µg / Kg day. Although these concentrations do not pose any risk to human health, the use of organic ingredients such as those analysed in our study would decrease in at least three times the risk. This is a remarkable effect considering the high toxicity of Cd on a population group as vulnerable as the infant population.

4. Conclusions

Weaning foods analyzed in the present study showed extremely low Cd concentrations, with values that do not reach 3% of tolerable weekly intake in the worst case. This reveals that using organic ingredients in the formulation of recipes of baby foods could be one of the necessary commitments to reduce the presence of this heavy metal in diet. On the other hand, the analysed organic jars did not represent a significant

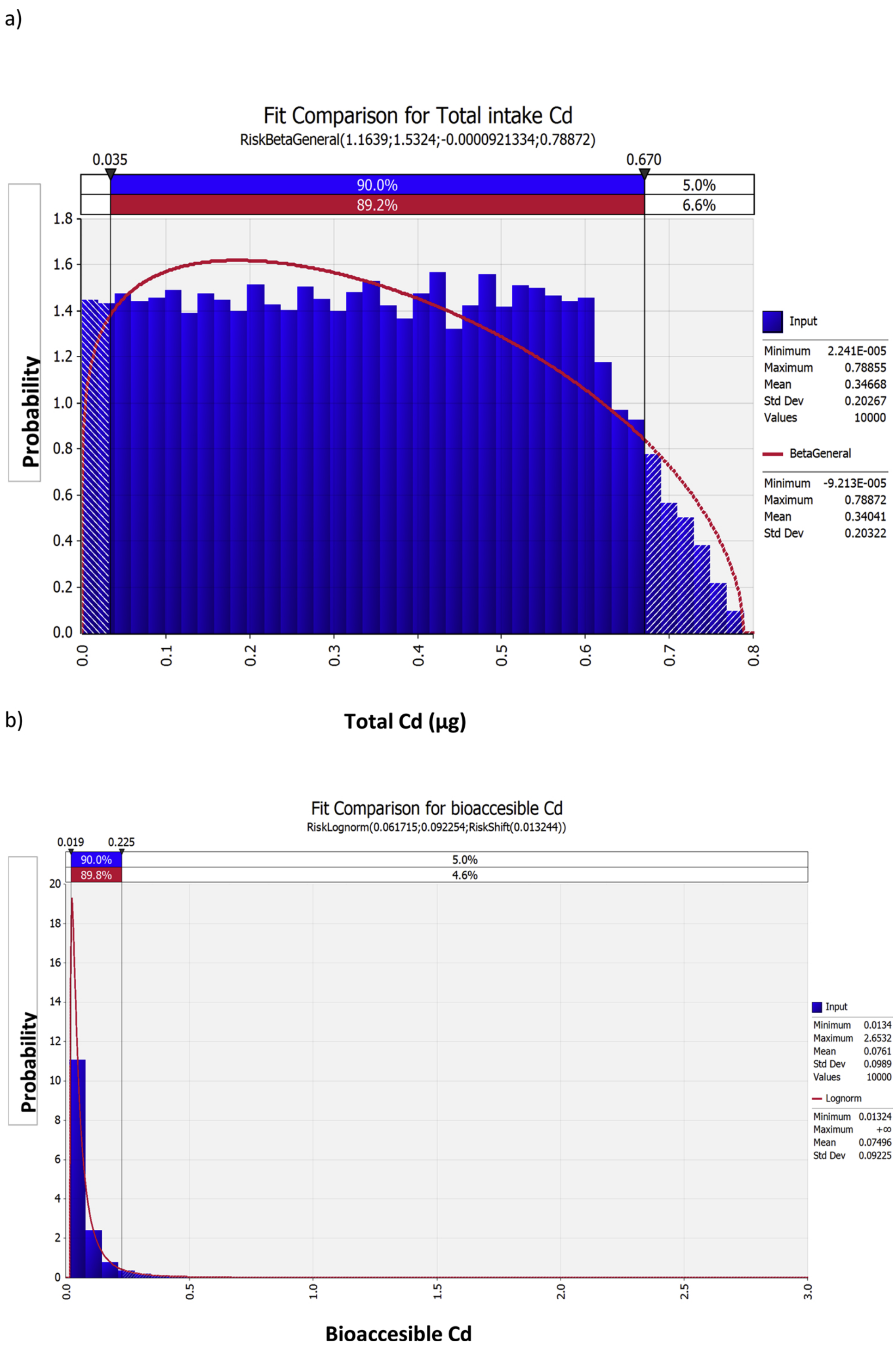

Fig. 2. Simulated data and fitted probabilistic distribution for Cd total trace and bioaccessible.

source of Se. The probabilistic assessment developed, showed that contributions to DRI of Se for infants 1–3 years old by consumption of these weaning foods, are excessively low (15% at best). No clear evidence can be established that this is due to the presence of organic ingredients. However, considering the importance of this micronutrient as one of the main antioxidant in human nutrition, strategies aimed at increasing the content and bioaccessibility of this element in these infant foods should be encouraged. Finally, a negative interaction with positive effects between fat content and Cd bioaccessibility was observed in weaning foods. An antagonistic and equal effect between Se and Cd was also observed. These findings reinforce the protective role of Se against heavy metal toxicity.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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