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Manganese levels in infant formula and young child nutritional beverages in the United States and France: Comparison to breast milk and regulations

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Abstract

Exposure to high levels of manganese (Mn) in children has recently been associated with adverse neurodevelopmental effects. Current infant formula regulations for Mn content were set between 1981 (United States), 2006 (European Union, France), and 2007 (Codex Alimentarius) prior to the publication of much of the growing body of research on the developmental neurotoxicity of Mn. In this study, we sought to measure the concentrations of Mn in some infant formulas and young child nutritional beverages available on the United States (US) and French markets using ion beam analysis by particle induced X-ray emission (PIXE) spectrometry and then compare the analytical results to concentrations reported in the literature for breast milk and applicable infant formula regulations and guidelines. We were particularly interested in measuring Mn concentrations in product types for which there is very little data from previous surveys, especially soy-based, rice-based, goat-milk based, chocolate-flavored, and nutritional beverages for young children that are not regulated as infant or follow-on formulas (e.g. "toddler formulas" and "toddler powders"). We purchased 44 infant formulas and young child nutritional beverage products in the US and France with varying protein sources (cow-milk, goat-milk, soy, rice) labelled for birth to 3 years. We selected these samples using maximum variation sampling to explore market extremes to facilitate comparisons to regulatory limits. Since this sampling method is non-probabilistic, other inferences cannot be made beyond this set of samples to the overall markets. We used ion beam analysis to measure the concentrations of Mn in each product. The range of measured Mn concentrations in the products is 160–2,800 μg/L, substantially higher than the 3–6 μg/L mean Mn concentration reported in human breast milk. All products satisfied national and Codex Alimentarius Commission (CAC) international standards for minimum Mn content in infant formulas; however, 7/25 of the products purchased in the US exceeded the CAC Guidance Upper Level of 100 μg Mn/kcal for infant formula.

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Abbreviations: μg, microgram; μL, microliter; μm, micrometer; ^{241}Am, the 241 isotope of americium; C, Celsius; Ca, calcium; CAC, Codex Alimentarius Commission; CENBG, Centre d'Etudes Nucléaires de Bordeaux Gradignan; CFR, Code of Federal Regulations; Cl, chlorine; cm, centimeter; CNRS, Centre National de la Recherche Scientifique; Cu, copper; e^-, electron; EC, European Commission; EU, European Union; eV, electron volt; FAO, Food and Agriculture Organization; FDA, Food and Drug Administration; Fe, iron; FWHM, full width at half maximum; g, gram; GRAS, generally recognized as safe; GUL, Guidance Upper Level; H^+, proton; IN2P3, Institut National de Physique Nucléaire et de Physique des Particules; K, potassium; kcal, kilocalorie; keV, kiloelectron volt; kg, kilogram; kj, kilojoule; L, liter; Max, maximum; MeV, megaelectron volt; mg, milligram; Min, minimum; mL, milliliter; mm^2, square millimeter; Mn, manganese; MR, magnetic resonance; N, nitrogen; n, number of samples; Na, sodium; NIST, National Institute of Standards & Technology; O, oxygen; P, phosphorus; pA, picoampere; PIXE, particle

Introduction

Manganese (Mn) is both an essential nutrient and a toxic element. The essentiality of Mn is reflected in national and international infant formula and food policies, which stipulate minimum Mn concentrations [1–3]. While Mn toxicity due to occupational exposures has been known for almost 200 years [4], the developmental neurotoxicity of Mn has only recently begun to be explored [4–8]. Infant formula regulations have not yet been adjusted to reflect this growing body of research on the developmental neurotoxicity of Mn; while maximum Mn content is regulated for infant formulas in some jurisdictions such as the European Union and France [3,9], in other jurisdictions, such as the United States (US), there is no regulatory maximum for Mn in infant formulas [1].

Serious concerns have recently been raised about relatively high Mn exposures and possible associated adverse effects on child neurodevelopment. Children exposed to higher levels of Mn compared to other children have been found to have impaired cognitive development [4,5,7,10–21] lower IQ or intelligence scores [4,6,7,10,12,13,22–31], impaired memory function [4, 6,10,24,32–37], lower academic skills or achievement [5,6,25], impaired executive function [6,10,12,24,32], lower visual-spatial ability [32,38], impaired motor function [4–6,24,25,36,39–41], impaired olfactory function [5,6,24], atypical brain structure or function [42,43], and relatively high Mn exposures are suspected of increasing the risk of attention deficits, hyperactivity, or attention deficit hyperactivity disorder (ADHD) [5–7,12,13,24,33,36,44], and other behavior and attention problems [4,6,7,12,16,25,45–47]. Some links that have been reported between high Mn levels and certain neurodevelopmental effects are not fully conclusive. Levels of Mn exposure that constitute excess for infants or young children as demonstrated by adverse health effects have not yet been rigorously identified in the literature; however, a benchmark dose for Mn in drinking water associated with decreased IQ has recently been calculated for school-aged children [22]. Many studies [13–21,26–43,45–47] and reviews [4–7, 10–12,22–25,44] of Mn exposures in children have found significant associations between higher Mn exposures and adverse neurodevelopmental outcomes. In contrast, some studies [48–52] and one review [53] have not found significant effects. The Mn concentrations found in infant formula and young child nutritional beverages in this study have not been compared to Mn intakes associated with adverse effects on child neurodevelopment.

In infants younger than weaning age (4–6 months), breast milk or infant formula typically constitutes the sole source of nutrition. Human breast milk is considered to be the optimal food for infants, providing all necessary macro- and micro-nutrients in sufficient quantities to sustain health and development through the age of weaning [54]. Breast milk usually contains 2–6 μg/L of Mn [55]. Infant formula has been reported to contain 195 times more Mn than the levels usually found in breast milk [56–58].

There are very few data available on Mn bioavailability in infants. In a study of 2–16 week-old infants, the retention of Mn was higher for formula-fed infants than for breast-fed infants due to the higher Mn content of infant formula [59]. It is noteworthy that in laboratory studies on Mn content in infant formula, high purity water with very low Mn content is used to reconstitute the infant formula. However, if infant formula is reconstituted with tap water, Mn from the tap water may further increase the total Mn content of the formula and the total Mn intake for the infant [56]. Moreover, Mn absorption rates are higher in neonates, 16–37% [59], compared to roughly 3% in adults [7]. Infants and especially neonates are further susceptible to Mn toxicity due to transiently diminished biliary excretion, which is the major route of Mn excretion in humans [7]. Overall, these data suggest that Mn intake and retention in children fed with infant formula are much higher than in children fed with breast milk or in adults.

induced X-ray emission; Rb, rubidium; RBS, Rutherford backscattering spectrometry; S, sulfur; s, sample standard deviation; Si, silicon; Si(Li), lithium-drifted silicon x-ray detector; Sr, strontium; SRM, Standard Reference Material; US, United States; WHO, World Health Organization; x, sample mean; Zn, zinc.

In this study, we sought to measure the concentrations of Mn in infant formulas and young child nutritional beverages available on the US and French markets using ion beam analysis by particle induced X-ray emission (PIXE) spectrometry and then compare the analytical results to concentrations reported in the literature for breast milk and applicable child feeding regulations and guidelines. Because of their wide availability in analytical chemistry laboratories, especially in industry, ICP-AES (inductively coupled plasma-atomic emission spectrometry) and FAAS (flame atomic absorption spectrometry) are the official methods for Mn analysis in infant formula [60,61]. Other analytical methods can be used as well, among them PIXE analysis [62–69], a less widespread technique that requires a particle accelerator. PIXE is a multi-elemental analytical technique that can be performed to quantify trace element content in a large variety of samples, including food samples, with minimum sample processing and high analytical accuracy.

Previous surveys of Mn concentrations in infant formulas often report higher Mn content in soy-based formulas than milk-based samples [56,58,70] but with few soy-based samples tested. In the present study, we were particularly interested in measuring Mn concentrations in product types for which there is very little data from previous surveys, especially soy-based, rice-based, goat-milk based, chocolate-flavored, and nutritional beverages for young children that are not regulated as infant or follow-on formulas (e.g. "toddler formulas" and "toddler powders"). Our hypothesis was that the Mn concentrations would be within the limits of infant formula regulations but might differ from the reported ranges in breast milk and that the protein source of the products might affect Mn content. We did not test the link between high Mn intake in children and brain disorder and adverse neurodevelopmental effects.

Material and methods

Selection of samples

A total of 25 products were purchased in the United States (US). Seventeen of these 25 products were labelled for use by infants (ages 0–12 months) [1] and 8 of these 25 products were labelled for use by young children (ages 1 year and older). Fifteen of the 17 infant products were standard infant formulas, and 2 of the 17 products were special medicinal "exempt infant formulas" [1]. Of the 8 products labelled for use by young children, 5 were "toddler" products, 2 were medical complete nutritional beverages, and 1 was a follow-on formula [2]. Of the 25 products obtained from the US market, 13 were cow milk-based, 5 were soy protein-based, 3 were goat-milk based, 3 were amino acid-based, and 1 was rice-based.

A total of 19 products were purchased in France. Seventeen of these 19 products were standard infant or follow-on formulas and 2 of these 19 products were liquid infant complementary foods [3,9]. The 2 liquid complementary foods from France were selected for this study in order to include samples containing chocolate or rice from the French market. In general, it was much more difficult to find formulas containing chocolate or rice in the French market than in the US market. We were not able to locate any powdered formula products in the French market that were chocolate flavored or rice-based. We were not able to obtain a soy-based or amino acid-based powdered formula in France.

For the purpose of this paper, all products labeled for use by young children 1 year and older, including follow-on formulas, medical complete nutritional beverages, liquid complementary foods, and "toddler" formulas, powders, or beverages are termed "young child nutritional beverage products".

All 44 of these samples were purchased in the US and France using maximum variation sampling [71]. More specifically, these samples were purposively selected to yield a wide range of Mn concentrations. That is, these samples were selected to contain supplemental Mn, soy,

rice, cow milk, goat milk, and chocolate, and to cover a wide range of ages, from birth to 3 years. This sampling method was designed to explore market extremes as well as apparently typical products in order to facilitate comparisons to regulatory compliance. Study goals were to determine the high and low ranges of Mn content in formula products rather than overall market trends. Since the sampling method is non-probabilistic, other inferences cannot be made beyond this set of samples to the overall market [71]. This sampling method was not designed to evaluate the batch to batch variability of any individual product.

Preparation of samples

Forty-two samples were powdered products and 2 samples were solutions. Solutions and suspensions (of the partially soluble products) of all 42 powdered samples were prepared for analysis using laboratory-grade plasticware. One solution or suspension was made for each sample of powdered product. A step-by-step protocol of infant formula sample preparation for PIXE/RBS determination of element concentrations has been deposited in the protocol.io repository [dx.doi.org/10.17504/protocols.io.5qxg5xn]. More specifically, between 1.0000 grams (g) and 1.0099 g of powdered product, and 10.00 milliliters (mL) of ultra-trace elemental analysis grade water (Fisher Chemical, Catalog No. W9-500) were delivered to a centrifuge tube, mixed, and stored at 4˚Celsius (C) until spotted on polycarbonate sample holders for ion beam analysis by PIXE spectrometry. The PIXE method can be applied to quantify trace elements in food samples [62]. PIXE has also been used to study trace element content in human breast milk and infant formulas [63–69].

Each of the 42 sample solutions or suspensions and each of the 2 liquid samples was mixed and spotted in 3 different locations on a single sample holder. Each spot was delivered with a 1.0 microliter (μL) air displacement pipet. These spots were dried at room temperature in a laminar flow hood. This process was repeated until a total of 3 spots were superimposed on each of the 3 different locations of each sample holder.

PIXE/RBS analysis

Each of the 3 spots on the sample holders was analyzed by PIXE; the 3 PIXE measurements were averaged to give a single reported concentration for each sample. These PIXE analyses were performed at the *Centre d'Etudes Nucléaires de Bordeaux Gradignan* (CENBG; Nuclear Studies Center of Bordeaux Gradignan) using the high-resolution beamline at the *Applications Interdisciplinaires de Faisceaux d'Ions en Région Aquitaine* (AIFIRA; Interdisciplinary Applications of Ion Beams in the Aquitaine Region) facility [72]. A Singletron particle accelerator system (High Voltage Engineering Europa B.V.) delivered a 3.0 megaelectron volt (MeV) proton (H^+) beam at approximately 300 picoamperes (pA) to the dried sample.

The principle of PIXE analysis as pioneered by Johansson and Johansson [73] is based on the interaction of accelerated charged particles, usually H^+, with the sample. In brief, the H^+ beam can ionize inner shell electrons from the atoms in the sample. For a specific element, an x-ray with a characteristic energy is emitted when an electron from an outer shell replaces an ionized electron from an inner shell. For elements heavier than sodium (Na), these x-rays were measured and averaged by 2 identical lithium-drifted silicon x-ray detectors, Si(Li). Each of these 2 detectors was at a 45˚ angle to the H^+ beam; that is, the H^+ beam bisects a 90˚ angle between the 2 detectors and the sample at the vertex [74]. Each detector was 2 cm from the sample. A 100 μm thick carbon foil funny filter, a filter with a pinhole drilled at its center, was placed between the sample and each of the 2 x-ray detectors. This attenuates the signal of the more abundant light elements and decreases the detector's dead time. Both Si(Li) x-ray detectors had a 145 electron volt (eV) energy resolution at 5.92 kiloelectron volt (keV), the Mn

KL2,3 x-ray emission line. This energy resolution enables the clear identification of the Mn KL2,3 x-ray emission line for the accurate quantification of Mn. For quantitative analysis of PIXE data, the system is calibrated with a set of thin film standards containing certified concentrations of reference elements (Micromatter™). Micromatter™ standards consist of the certified elements, each with an areal density of about 50 μg/cm^2, deposited on a 6 mm Mylar foil. PIXE allows the determination of element concentration expressed in terms of the areal mass of the element (μg/cm^2).

In order to quantify the element concentration in terms of μg of the element per g of sample, a second ion beam analysis technique, Rutherford backscattering spectrometry (RBS), is carried out simultaneously to PIXE [74,75]. For the RBS analysis, a fraction of the protons from the H^+ beam is repelled by the positively charged atomic nuclei in the sample [76]. The energies of the backscattered protons were measured with a 25 mm^2 silicon passivated detector, 12 keV full width at half maximum at 5.486 MeV using the 241 isotope of americium (^{241}Am). This detector was at a 45˚ angle to the H^+ beam; that is, the H^+ beam forms a 45˚ angle to the detector with the sample at the vertex [74]. This detector was 2 cm from the sample. RBS measures the atomic concentrations of carbon (C), nitrogen (N), and oxygen (O), the main components in biological samples that cannot be measured by PIXE, which is sensitive to elements of atomic number > 11. Therefore, for sample matrixes, such as milk, which are composed mainly of light elements, RBS allows the determination of the total areal mass of the sample expressed in g/cm^2. The combination of PIXE and RBS allows the quantification of element concentrations in μg/g of dry mass.

In addition, RBS measures the number of H^+ that interact with the sample during the analysis; this parameter is required to quantify the concentrations of the elements that are detected by PIXE [74]. Therefore, the simultaneous analysis of PIXE and RBS enables the quantification of the concentrations of elements in solid samples [74,75,77]. GupixWin software [78] and SIMNRA software [79] were used to calculate these concentrations.

The accuracy of PIXE/RBS quantitative analysis was assessed with the analysis of a National Institute of Standards & Technology (NIST) Standard Reference Material® (SRM), 1849a Infant/Adult Nutritional Formula. This assessment was used to validate the entire analytical procedure for Mn quantification, from sample preparation through data treatment.

The measured concentrations were converted to μg of element per liter (L) of prepared product following the instructions from the manufacturer's labels. This was done using the mass of solid product measured 3 times to the nearest 0.0001 g with an analytical balance, and the final volume of the prepared formula measured 3 times to the nearest 0.1 mL with a graduated cylinder. For formula sold in the US, the legal definition of 1 fluid ounce equals 30 mL was used for this preparation since product labels must use this definition when reporting element concentrations [80].

Finally, the measured concentrations were converted to μg of element per 100 kilocalories (kcal) of prepared product. This was done using the reported kcal/mass of dried product or final volume of prepared product from the manufacturer's label.

Results and discussion

The analysis of Standard Reference Material® by particle induced x-ray emission, Rutherford backscattering spectrometry

National Institute of Standards & Technology (NIST) Standard Reference Material® (SRM) 1849a, Infant/Adult Nutritional Formula is the current industry standard for testing infant formula in the United States [81]. In this study, NIST SRM 1849a Infant/Adult Nutritional Formula was analyzed 3 times to assess the accuracy of PIXE/RBS for measuring the

concentrations of representative elements in infant formulas and young child nutritional beverage products. NIST SRM 1849a was processed in exactly the same manner as the samples of infant formula and young child nutritional beverages in this study. Relative error was used to assess this accuracy (see Eq 1) [82].

$$\text{Relative Error} = E_r = \frac{(\bar{x} - x_t)}{x_t} \times 100\% \quad (1)$$

The certified mass fraction value for manganese in NIST SRM 1849a is 49.59 ± 0.97 μg/g. The mean and sample standard deviation for the 3 analyses of Mn by PIXE are 49.48 ± 1.61 μg/g, respectively. In this case, $\bar{x}$ is the sample mean for Mn in μg/g, and x_t is the true or certified mass fraction for Mn in μg/g. The relative error for Mn was -0.22%. This -0.22% relative error and 1.61 μg/g sample standard deviation for the NIST SRM are estimates of the accuracy and precision for the measurement of Mn in each container of sample in this study, respectively.

NIST SRM 1849a contains more Mn than the infant products in this study; the certified mass fraction is 49.59 ± 0.97 μg/g for SRM 1849a, and mass fractions ranged from 1.3 μg/g to 32 μg/g for all the samples in this study. This might be a result of SRM 1849a Infant/Adult Nutritional Formula being designed by NIST to test both infant and adult nutritional formula, instead of just infant formula. Regardless, SRM 1849a is the current industry standard for testing infant formula in the United States [81]. Therefore, we used the same standard of comparison as industry laboratories use for product labeling under the Infant Formula Act of 1980.

The concentrations of manganese in infant formulas and young child nutritional beverage products from the US and French markets

The measured concentrations of Mn in each of the 25 samples from the US market are shown in Table 1, along with the minimum, maximum, number of samples, the protein source, the labeled age range of the product, and whether the product was labeled for special medical purposes or contained chocolate. These parameters for the 19 samples from the French market are shown in Table 2.

The concentrations in Tables 1 and 2 are given in 3 equivalent units, μg of element per g of dried product (μg/g), μg of element per L of prepared product (μg/L), and μg of element per 100 kcal of prepared product (μg/100 kcal). In prior surveys of infant formulas and cow, goat, and human milks, a vast array of units, such as μg/quart [83] and nanomoles per L [84] have been used, requiring unit conversions in order to compare results across studies. In this study, results are reported in μg/g because these units are directly measured by PIXE/RBS and many other instrumental methods.

In this study, results are also reported in μg/L because these units are readily comparable to many prior surveys of liquid milks [85–87]. The μg/L were calculated from the measured μg/g by PIXE/RBS and the measured mass of dried product that was used to make a measured final volume of prepared product according to the instructions on the product labels. Each measurement was done 3 times and averaged; each average was used to convert μg/g to μg/L.

In this study, results are also reported in μg/100 kcal because these units are used in policies and regulations [1,2,88–91]. The regulations refer to energy units (kcals) so that they automatically scale to an infant's weight and total energy requirements. Units given by kcals can be readily used to tabulate total intake of nutrients in both single-item and mixed diets that include both solid and liquid foods. The μg/100 kcal were calculated from the measured μg/g by PIXE/RBS and the reported kcal/mass of dried product or final volume of prepared product from the manufacturer's label.

Table 1. Mn content by PIXE/RBS spectrometry for the samples from the US market.

ID	Mn (μg/g)	Mn (μg/L)	Mn (μg/100 kcal)	Supplemental Mn	Protein Source	Chocolate	Labeled Age Range	Medical
US01	1.8	230	36	No	Cow[a]	No	infant	No
US02	2.7	400	55	No	Cow[a]	No	6 months +	No
US03	2.3	310	47	Yes	Cow[a]	No	0–12 months	No
US04	1.3	160	26	No	Cow	No	toddler	No
US05	7.9	1,000	170	No	Soy	No	toddler	No
US06	3.4	430	65	Yes	Cow	No	0–12 months	No
US07	2.7	320	48	Yes	Cow[a]	No	infant	No
US08	7.4	1,600	160	Yes	Amino acids	No	1+ years	Yes
US09	2.6	340	50	Yes	Goat	No	1–3 years	No
US10	11	2,100	240	Yes	Cow	Yes	1–13 years	Yes
US11	9.2	1,100	220	No	Goat	Yes	13 months-8 years	No
US12	32	2,800	860	Yes	Rice	No	1–4 years	No
US13	2.2	330	47	Yes	Goat	No	1–2 years	No
US14	5.7	830	120	Yes	Amino acids	No	infant	No
US15	4.2	540	81	No	Soy	No	infant	No
US16	3.9	480	72	No	Soy	No	infant	No
US17	2.9	420	64	No	Soy	No	0–12 months	No
US18	1.6	210	31	Yes	Amino acids	No	0–12 months	No
US19	6.3	790	120	Yes	Soy	No	0–12 months	No
US20	1.6	230	34	Yes	Cow	No	infant	No
US21	2.7	340	51	Yes	Cow	No	infant	No
US22	2.9	420	63	Yes	Cow	No	infant	No
US23	2.1	260	39	Yes	Cow	No	infant	No
US24	2.5	320	48	Yes	Cow	No	infant	No
US25	2.1	260	39	Yes	Cow	No	infant	No
Min	1.3	160	26					
Max	32	2,800	860					
Mean[b]	**5.0**[b]	**650**[b]	**110**[b]					
Median[b]	**2.7**[b]	**400**[b]	**55**[b]					
***s*[b]**	**6.2**[b]	**650**[b]	**170**[b]					

a Contains rice

b Applies to this dataset only. Since the sampling method was non-probabilistic, inferences to the overall market are not intended and should not be made.

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Ingredients and the concentrations of manganese in infant formulas and young child nutritional beverage products

The total Mn concentration of an infant formula or young child nutritional beverage product includes any supplemental Mn, if added by the manufacturer, and the Mn from all of the other ingredients. Manganese is often added by the manufacturer to ensure that the Mn concentration of the final product is greater than the minimum required Mn concentration set by regulations. If a product is supplemented with Mn, then it is impossible to assign the total Mn concentration of the final product to any given ingredient, such as soy, rice, cow milk, goat milk, or chocolate. Therefore, all products with supplemental Mn in our survey were classified as "supplemented" for the purpose of assessing the effect of major ingredients on the total Mn concentration of each product. Products without supplemental Mn that contained soy, rice, cow milk, or chocolate were classified as "soy", "rice", "cow milk", or "chocolate", respectively.

Table 2. Mn content by PIXE/RBS for the samples from the French market.

ID	Mn (μg/g)	Mn (μg/L)	Mn (μg/100 kcal)	Supplemental Mn	Protein Source	Chocolate	Labeled Age Range	Medical
FR01	1.7	240	35	Yes	Cow	No	0–6 months	No
FR02	2.1	290	46	Yes	Cow	No	12+ months	No
FR03	1.7	230	37	Yes	Cow	No	6–12 months	No
FR04	2.3	290	46	Yes	Goat	No	6+ months	No
FR05	1.7	220	34	Yes	Goat	No	1+ years	No
FR06	2.4	310	46	Yes	Cow	No	0–6 months	No
FR07	2.7	350	53	Yes	Cow	No	0–6 months	No
FR08	5.9	1,200	140	No	Cow	Yes	6+ months	No
FR09	4.6	860	100	No	Cow[a]	No	6+ months	No
FR10	2.5	350	53	Yes	Goat	No	6+ months	No
FR11	2.0	320	42	Yes	Cow	No	6–12 months	No
FR12	2.6	390	55	Yes	Cow	No	10+ months	No
FR13	1.5	200	32	Yes	Cow	No	1–3 years	No
FR14	2.1	300	44	Yes	Cow	No	6–12 months	No
FR15	2.0	280	40	Yes	Cow[a]	No	6 months +	No
FR16	2.0	320	47	Yes	Cow	No	6–12 months	No
FR17	1.9	290	44	Yes	Cow	No	1+ years	No
FR18	2.5	340	52	Yes	Cow	No	12+ months	No
FR19	4.4	560	93	Yes	Cow[a]	No	0–36 months	No
Min	1.5	200	32					
Max	5.9	1,200	140					
Mean[b]	**2.6**[b]	**390**[b]	**55**[b]					
Median[b]	**2.1**[b]	**310**[b]	**46**[b]					
s[b]	**1.1**[b]	**250**[b]	**27**[b]					

a Contains rice

b Applies to this dataset only. Since the sampling method was non-probabilistic, inferences to the overall market are not intended and should not be made.

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In the 44 samples that we tested, 4 were soy-protein based without supplemental Mn, 2 contained rice without supplemental Mn, 2 were cow milk-protein based without supplemental Mn, and 2 contained chocolate without supplemental Mn; the remaining 34 samples contained supplemental Mn (see Fig 1). The minimum, maximum, and number of samples (*n*) for supplemented and soy products are shown in Table 3; cow, rice and chocolate products are not included in Table 3 due to the low number of samples in these categories.

In this study, 34 of the 44 products listed supplemental Mn on the label (Fig 1 and Table 3); we classified these products as "supplemented". The addition of supplemental Mn by manufacturers is a possible reason why the largest maximum concentration of Mn in this study, 860 μg/100 kcal, is from a product that was supplemented with Mn (Fig 1 and Table 3). For comparison, a Mn concentration of 860 μg/100 kcal is about 1,000 times greater than that of breast milk, approximately 0.83 μg/100 kcal [92].

Four of the products in this study were not supplemented with Mn and used soy as a major ingredient (Fig 1 and Table 3). Soy has a relatively high native concentration of Mn [93]. Prior surveys have consistently shown that soy protein-based formulas have higher Mn concentrations than milk-based formulas [83,86,94–97]. Therefore, soy is a possible source of the relatively high maximum concentration of Mn in the 4 "soy" products, 170 μg/100 kcal (Fig 1 and Table 3). For comparison, a Mn concentration of 170 μg/100 kcal is about 200 times greater than that of breast milk [92].

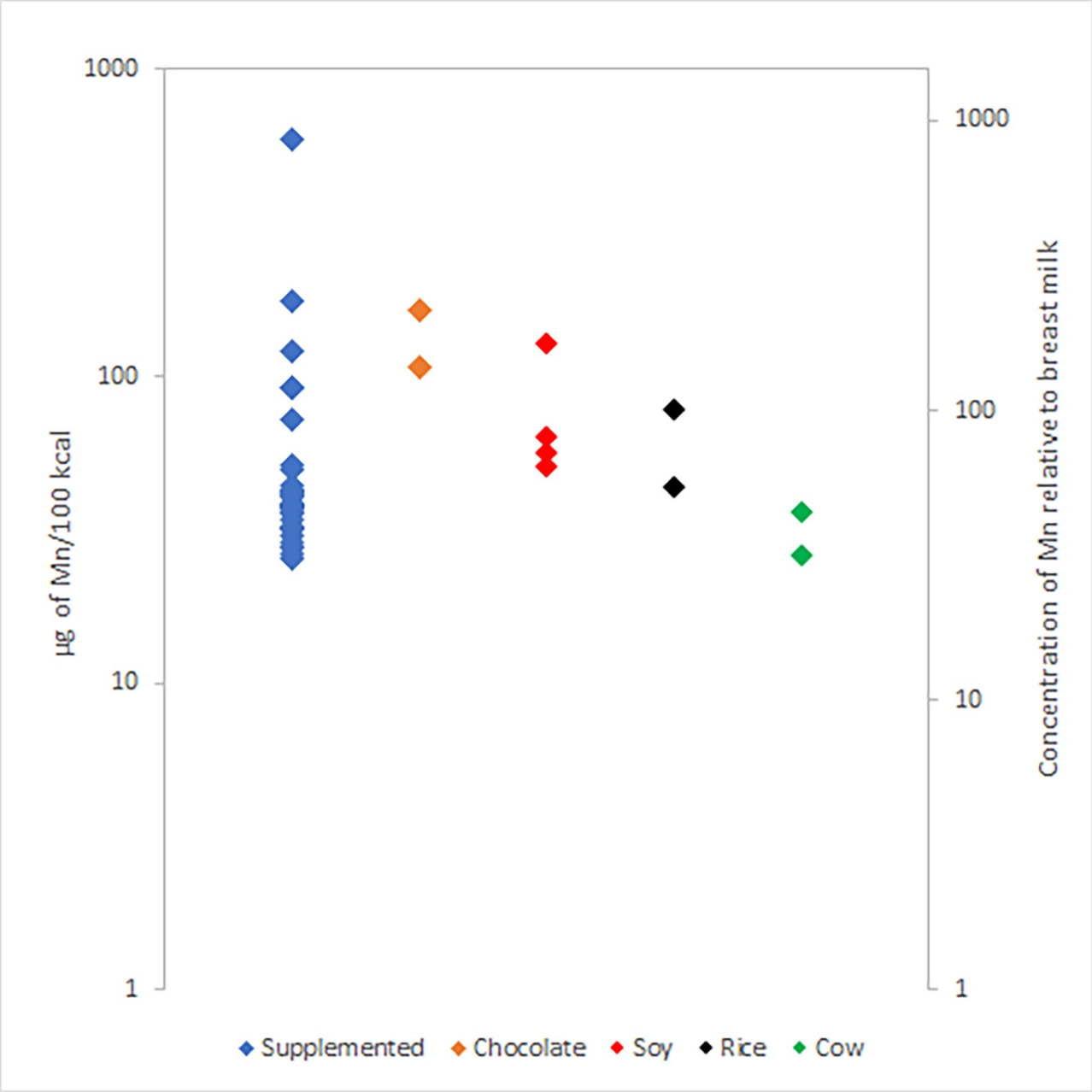

Fig 1. The minimum and maximum concentrations of Mn of prepared infant formulas and young child nutritional beverage products according to ingredient classes. These concentrations are also given relative to the concentration of Mn in breast milk [92].

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Two of the products in this study were not supplemented with Mn and used rice as an ingredient (Fig 1). Rice has a relatively high native concentration of Mn [98,99]. Rice is a possible source of the relatively high concentration of Mn in these "rice" products, with a maximum concentration of 100 µg/100 kcal. For comparison, a Mn concentration of 100 µg/100 kcal is about 120 times greater than that of breast milk [92].

Two of the products in this study were not supplemented with Mn and used cow milk as an ingredient (Fig 1). Cow milk generally has a much lower native concentration of Mn than soy, rice, or chocolate, but a higher concentration of Mn than human breast milk [55,94,95, 100–

Table 3. Ranges of Mn in samples according to ingredients. Samples with supplemental Mn.

Min	1.5 μg/g	200 μg/L	31 μg/100 kcal
Max	32 μg/g	2,800 μg/L	860 μg/100 kcal
$\bar{x}^a$	3.8 μg/g[a]	510 μg/L[a]	84 μg/100 kcal[a]
Median[a]	2.4 μg/g[a]	320 μg/L[a]	47 μg/100 kcal[a]
s^a	5.3 μg/g[a]	570 μg/L[a]	140 μg/100 kcal[a]
N	34 samples	34 samples	34 samples
Samples with soy protein and without supplemental Mn			
Min	2.9 μg/g	420 μg/L	64 μg/100 kcal
Max	7.9 μg/g	1,000 μg/L	170 μg/100 kcal
$\bar{x}^a$	4.7 μg/g[a]	620 μg/L[a]	96 μg/100 kcal[a]
Median[a]	4.1 μg/g[a]	510 μg/L[a]	77 μg/100 kcal[a]
s^a	2.2 μg/g[a]	280 μg/L[a]	47 μg/100 kcal[a]
N	4 samples	4 samples	4 samples

a Applies to this dataset only. Since the sampling method was non-probabilistic, inferences to the overall market are not intended and should not be made.

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107]. This is a possible reason why "cow milk" products had the lowest maximum concentration of Mn when compared to "soy", "rice", and "chocolate" products, 36 μg/100 kcal (Fig 1 and Table 3). For comparison, a Mn concentration of 36 μg/100 kcal is about 43 times greater than that of breast milk [92]. Similarly, the 2 "cow milk" products also had the lowest minimum concentration of Mn in this study, 26 μg/100 kcal (Fig 1 and Table 3). A Mn concentration of 26 μg/100 kcal is about 32 times greater than that of breast milk [92].

Two of the products in this study were not supplemented with Mn and used chocolate as an ingredient (Fig 1). Chocolate has a relatively high native concentration of Mn [98,104–107]. Thus, chocolate is a possible source of the relatively high maximum concentration of Mn in the 2 "chocolate" products, 220 μg/100 kcal. For comparison, a Mn concentration of 220 μg/100 kcal is about 260 times greater than that of breast milk [92].

In this study, one of the products based on goat milk did not have supplemental Mn; however, it also contained chocolate, so the effect of goat milk on total Mn concentration could not be assessed from this set of samples. Prior surveys suggest that the Mn concentration of goat milk is comparable to that of cow milk [100,108–110]; similarly, the Mn concentration of goat-milk based formulas has been shown to be comparable to that of cow-milk based formulas [96].

Targeted ages of consumption and the concentration of manganese in infant formulas and young child nutritional beverage products

The products that we tested were labeled for use by different ages. Products sold in the French market were all labeled with a number (1, 2, 3, or 4) to indicate the intended "age stage" of the product. Stage 1 products were labeled for ages 0–6 months (or up to 36 months for special medical formulas), stage 2 for ages 6–12 months (or older), and stages 3 and 4 for ages 1–3 years (or older). In this study, 4 samples were labelled for use by infants ages 0–6 months (stage 1), 9 were labelled for use by infants ages 6 months and older (stage 2), and 6 were labelled for use by young children ages 1 year and older (stages 3 or 4). According to French law [3], the term *préparation pour nourrissons* (infant formula) applies to products intended for the first months of life to satisfy all nutritional needs of a *nourrisson* [3] (infant, a child under 12 months old). In contrast, the term *préparation de suite* (follow-on formula), is

intended to be the primary liquid nutrition for a *nourrisson* (infant) who is beginning to include complementary foods in the diet. Thus, products labeled stage 1 would be classified as *préparations pour nourrissons*, products labeled stage 2 would be classified as *préparations de suite*. Products labeled stages 3 or 4 are for *enfants en bas âge* (young children, ages 1–3 years), and are also regulated by the French law regarding *préparations pour nourrissons et aux préparations de suite* (infant and follow-on formulas) [3]. The 2 liquid complementary foods that we tested from France were labeled for use by ages 6 months and older but are classified as foods, not infant or follow-on formulas according to French law [3].

Products that we tested from the US market were labeled "infant formula", "toddler formula", or "toddler powder". Some infant formula products purchased in the US that were produced outside the US also bore age stage numbers, similar to the products from France, in addition to the term "infant formula". In this study, 16 samples were labelled for use by infants ages 0 months and older, 1 was labelled for use by infants ages 6 months and older, and 8 were labelled for use by young children ages 1 year and older. In the US market, a product can only use the term "infant formula" on the label if it meets special Food and Drug Administration (FDA) requirements for infant formulas [111]. By definition, an infant formula must be a "simulation of human milk" or "a complete or partial substitute for human milk" [111]. The FDA does not distinguish between "infant formulas" and "follow-on" formulas. However, the FDA exempts certain infant formulas intended for special medical purposes (e.g. severe allergies) from standard infant formula regulations concerning nutrient contents [111]. By definition, "infants" are persons not more than 12 months old [112]. Notably, there is no legal definition of "toddler formula" or "toddler powder" in the US [112,113]. Products labeled "toddler formula" or "toddler powder" are presumably for children older than 1 year. They are covered by the regulations that apply to ordinary foods but are not subject to the special regulations for products labeled "infant formula" [112–114].

The minimum and maximum Mn concentrations for the 30 tested products intended for children less than 1 year old (products labeled stage 1 or 2 in France or "infant formula" in the US) were 1.56 μg/g and 6.32 μg/g, respectively. The minimum and maximum Mn concentrations for the 14 products intended for children over 1 year were 1.26 μg/g and 31.85 μg/g, respectively. Some researchers have argued that all infant formulas should be "staged" to better meet the changing nutritional requirements of growing children [115]; such staging would require a reassessment of the growing child's need for Mn from birth through the toddler years.

Comparison of manganese concentrations in infant formulas and young child nutritional beverage products to Mn concentrations in breast milk and daily manganese intakes

It is presently assumed that breast milk from healthy, well-nourished mothers supplies adequate amounts of macro- and micro-nutrients, including Mn, at least for the first 6 months of life [54]. A review of longitudinal studies of Mn concentrations in breast milk reported average concentrations of 3–6 μg/L 2–4 weeks post-partum, with individual values between 2–8 μg/L [55]. A recent study of the Mn content in breast milk from various geographic areas (Argentina, Namibia, Poland, and the United States) reported mean values of 2–11 μg/L with individual values ranging from 1 to 30 μg/L [116]. All of the products that we tested contained substantially higher Mn concentrations (US minimum: 160 μg/L; France minimum: 200 μg/L) than the maximum concentration reported for breast milk. Assuming a mean value for breast milk around 3 μg/L in the US and France [116], minimal Mn concentrations in infant formula are about 53 (US) to 67 (France) times higher, while maximal Mn concentrations are 930 (US)

and 400 (France) times higher. These ratios calculated from μg/L values are comparable with the ratios calculated from μg/100 kcal values (Fig 1). A substantially higher content of Mn in infant formula compared to breast milk has also been reported in all similar studies [56–58].

In a 2011 study of infant formulas in the Swedish market in which formulas were found to contain between 25–499 μg Mn/L, it was noted that daily Mn intakes of infants fed formula could be 114-fold higher than those of exclusively breast-fed infants [56]. The authors noted that "concentrations of several hundred μg/l, which we found in about half of the investigated formulas, may in fact not be safe for the infant" [56]. In the present study, in which the range of Mn content from formulas was 160–2800 μg Mn/L, daily intakes of Mn would be even higher than those discussed in the Swedish study [56].

Infant formula and follow-on/follow-up formula standards and regulations

The joint World Health Organization/Food and Agriculture Organization Codex Alimentarius Commission standards for manganese in infant formula and follow-up formula. The joint WHO/FAO Codex Alimentarius Commission (CAC) publishes standards for infant formulas, follow-up formulas and formulas for special medical purposes intended for infants [88,89]. For infant formula nutrients such as Mn, standards are stated in mass of nutrient/100 kilocalorie (kcal) of prepared formula to automatically scale to infant energy requirements according to infant body weight, to varying types of formula (powder or liquid), and to varying masses of powder used to prepare a volume of formula. The CAC states that infant formula prepared for consumption shall contain between 60 kcal (250 kJ) and 70 kcal (295 kJ) of energy per 100 mL, a minimum of 1 μg of Mn/100 kcal, and not exceed the Guidance Upper Level (GUL) of 100 μg of Mn/100 kcal [2, 88]. The CAC does not provide guidance for the Mn content of follow-on formulas [89].

In this study, the mean energy content of the 42 powdered products was $683._{7\ldots}$ kcal/L of prepared formula; therefore, the 1 μg of Mn/100 kcal minimum equals 6.84 μg of Mn/L of prepared product in this study as follows (see Eq 2; nonsignificant digits, such as 7. . ., are shown as a subscript followed by an ellipsis and are included in all steps of a calculation to prevent rounding error).

$$\text{Estimated Minimum Volume Basis} = \frac{1\ \mu\text{g of Mn}}{100\ \text{kcal}} \times \frac{683._{7\ldots}\ \text{kcal}}{\text{L of prepared product}} = \frac{6.84\ \mu\text{g of Mn}}{\text{L of prepared product}} \quad (2)$$

Similarly, this 100 μg of Mn/100 kcal GUL equals 684 μg of Mn/L of prepared product in this study as follows (see Eq 3).

$$\text{Estimated Guidance Upper Level Volume Basis} = \frac{100\ \mu\text{g of Mn}}{100\ \text{kcal}} \times \frac{683._{7\ldots}\ \text{kcal}}{\text{L of prepared product}} = \frac{684\ \mu\text{g of Mn}}{\text{L of prepared product}} \quad (3)$$

None of the 17 powdered infant or follow-on formulas purchased in France for this study had a Mn concentration greater than the CAC 100 μg of Mn/100 kcal GUL. One of the 2 liquid products, an infant complementary food, had a concentration of 140 μg of Mn/100 kcal. Complementary foods are not covered by the CAC Standard for Follow-Up Formula [89]. This sample was not supplemented with Mn but contained chocolate, which can have a large effect on the concentration of Mn (Fig 1 and Table 3) [98,104–107].

Two of the 16 infant formula products purchased in the US for this study had a Mn concentration that was greater than the CAC 100 μg of Mn/100 kcal GUL for infant formulas. One of

these products was a soy-based infant formula and the other was an amino acid-based medical infant formula. Five other products exceeded 100 μg of Mn/100 kcal but were labeled for children ages 12 months and older; the CAC standard does not stipulate a maximum Mn content for follow-up formulas [89]. The concentrations of Mn in the 7 US products with more than 100 μg of Mn/100 kcal ranged from 120 to 860 μg of Mn/100 kcal. Five of the 7 products that exceeded 100 μg of Mn/100 kcal contained supplemental Mn. Of the 2 products that exceeded 100 μg of Mn/100 kcal and did not contain supplemental Mn, 1 was soy-based and the other contained chocolate. These ingredients can have a large effect on the concentration of Mn (Fig 1 and Table 3) [93,98,104–107].

Three of the 7 products from the US market that exceeded the CAC 100 μg of Mn/100 kcal GUL for infant formula had "toddler" formula or powder on the manufacturer's label, not "infant formula". Similar to follow-up formulas, toddler formulas and toddler powders are marketed as milk substitutes for young children, but are not regulated as infant formulas, and their status with respect to the CAC standards is unclear [113,114,117,118]. The CAC states that "Follow-up formula is a food prepared from the milk of cows or other animals and/or other constituents of animal and/or plant origin, which have been proved to be suitable for infants from the 6th month on and for young children", where young children are defined as "persons from the age of more than 12 months up to the age of three years (36 months)" [89]. By these CAC definitions, products labelled "toddler formula" would be classified as "follow-up" formulas, except they have not "been proved to be suitable for infants from the 6th month on and for young children" [89]. Terms such as "toddler formula", "toddler powder", or "toddler beverage" on labels may suggest to parents that these beverages can be used similar to infant or follow-on formulas [113,114,117,118]. Moreover, the WHO has observed, "It is clear that the marketing of toddler milks is a response to legislation that restricts marketing of formulas to infants" and emphasizes "the now common cross-promotion practice by which breast-milk substitutes for infants are promoted through labelling and advertisements of toddler formulas is a threat to breastfeeding and infant health" [118].

The European Union (EU) and the Republic of France regulations for manganese in infant formula and follow-on formula. The European Parliament regulates infant formulas and follow-on formulas within the European Union [9,90] while the the *République Française* (Republic of France) publishes regulations covering *préparations pour nourrissons et aux préparations de suite* (infant formulas and follow-on formulas) in France [3]. The current EU and French regulations for infant and follow-on formulas stipulate a minimum content of 1 μg of Mn/100 kcal and a maximum content of 100 μg of Mn/100 kcal [3,9].

In this study, all of the 17 infant or follow-on formulas purchased in France contained more than 1 μg of Mn/100 kcal of prepared product. None of the infant or follow-on formulas purchased in France had a measured Mn concentration that was greater than the 100 μg of Mn/100 kcal Maximum allowed by French law [3].

The concentrations of Mn in the 17 infant or follow-on formulas purchased in France ranged from 32 to 93 μg of Mn/100 kcal. The product with 93 μg of Mn/100 kcal has about 110 times more Mn than breast milk [92]. This product contained supplemental Mn. The supplementation of infant formula with Mn is allowed by the Republic of France [3]. In this study, 17 of the 19 products that we purchased in France were supplemented with Mn. The list of *sels autorisés* (allowed salts) is manganese carbonate, manganese chloride, manganese citrate, manganese sulfate, and manganese gluconate [3]. In 2002, the *Agence française de sécurité sanitaire des aliments* (AFSSA; French Food Safety Agency) stated "*l'enrichissement d'une préparation de suite en manganèse n'a aucune justification nutritionnelle* (the enrichment of follow-on formula with manganese does not have any nutritional justification)" [119].

The Food and Drug Administration and United States nutritional standards for manganese in infant formula. The Federal Food, Drug, and Cosmetic Act passed by the US Congress regulates infant formulas in the US [1]. According to the Federal Food, Drug, and Cosmetic Act, the Minimum Level for Mn in prepared infant formula is 5 μg/100 kcal, and no Maximum Level is specified [1].

In the 42 powdered products that we tested, which had a mean energy content of 683.$_{7...}$ kcal/L of prepared product, 5 μg of Mn/100 kcal would correspond to approximately 34 μg of Mn/L when prepared according to labelled instructions (see Eq 4).

$$\begin{matrix}\text{Estimated}\\ \text{Minimum Level}\\ \text{Volume Basis}\end{matrix} = \frac{5\ \mu\text{g of Mn}}{100\ \text{kcal}} \times \frac{683._{7...}\ \text{kcal}}{\text{L of prepared product}} = \frac{34\ \mu\text{g of Mn}}{\text{L of prepared product}} \quad (4)$$

All of the products (25 out of 25) purchased in the US for this study had a measured Mn concentration that was greater than the 5 μg of Mn/100 kcal FDA Minimum Level for infant formulas. The concentrations of Mn in these 25 products ranged from 26 to 860 μg of Mn/100 kcal. The product with 860 μg of Mn/100 kcal has about 1,000 times more Mn than breast milk [92]. This product was labeled "toddler powder", so it is not regulated by US laws regarding infant formula [113,114].

Sixty-eight percent (17 out of 25) of the products we purchased on the US market were supplemented with Mn. In 1985, manganese chloride, manganese citrate, manganese gluconate, and manganese sulfate were "generally recognized as safe (GRAS) as a direct human food ingredient" and approved as sources of Mn for use in "infant formulas in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act" [120]. By definition, GRAS is "A food substance that is not subject to premarket review and approval by FDA because it is generally recognized, by qualified experts, to be safe under the intended conditions of use" [121].

Study limitations

By design, the maximum variation sampling method for this study focused on the margins of the markets, to identify products that might potentially have either too little or too much manganese to satisfy regulatory requirements. Consequently, no inferential statistics can be made about the markets as a whole from our results, and the only statistics that can be compared across the French and US markets are maxima and minima; measures of central tendency, sample variation, and tests of statistical significance concerning market differences cannot be performed with these data. In addition, our selection of products according to maximum variation sampling relied on labeled Mn content and ingredients such as soy, rice, cow milk, goat milk, and chocolate, since we predicted these factors to influence Mn content; however, actual Mn content may not be accurately labeled or may be determined by other factors. For this reason, we included multiple examples of each type of product that we predicted were likely to have very high or very low Mn content. Widespread Mn supplementation of products limited our efforts to determine whether specific ingredients such as soy protein, rice, cow milk, goat milk, or chocolate might be associated with high or low Mn content since it was difficult to find sufficient numbers of unsupplemented products of specific types. Furthermore, this study considers only whether products meet regulatory requirements and guidelines; it does not directly examine the question of whether feeding with these products would lead to either adequate or excess intakes of Mn, or the link between high Mn intake in children and brain disorder and adverse neurodevelopmental effects.

Conclusions

In this study we used simultaneous particle induced X-ray emission (PIXE) and Rutherford backscattering (RBS) spectrometry to measure the concentration of Mn in μg of element per g of dried product for a selection of infant formulas and young child nutritional beverage products purchased in the US and France. The accuracy of PIXE/RBS for measuring Mn was assessed by the analysis of a National Institute of Standards & Technology (NIST) Standard Reference Material®, 1849a Infant/Adult Nutritional Formula; the relative error for the measurements of Mn was -0.22%. All 44 of the samples we analyzed had measurable concentrations of Mn.

In general, products with supplemental Mn had higher Mn concentrations than products without supplemental Mn (Fig 1 and Table 3). For products without supplemental Mn, products with chocolate had the most Mn, followed by soy, rice, and cow milk, all of which had more Mn than reported concentrations in breast milk (Fig 1 and Table 3).

The ranges of concentrations of Mn in the infant formulas and young child nutritional beverage products greatly exceeded the ranges of Mn concentrations reported in breast milk. The ranges of Mn concentrations in the 17 infant and follow-on formula products purchased in France were in compliance with French, European, and international standards. The ranges of Mn concentrations in the 25 infant formula and young child nutritional beverages purchased in the US satisfied the US minimum standards for infant formula. While 2 of the 16 US infant formula products and 5 of the 8 products labeled for children over 12 months that we tested exceeded the CAC international Guidance Upper Level (GUL) of 100 Mn/100 kcal for infant formulas, US laws do not currently stipulate a maximum Mn content for infant formulas or regulate Mn content in formulas or powders for children over 12 months old and the CAC does not stipulate a GUL for Mn for formula products for children over 12 months old 89].

Given the recent research demonstrating adverse effects of excess Mn exposure for neurodevelopment [4–7,10–47], stricter upper limits for Mn content in infant formulas should be considered by regulators. The 38-year-old requirement for minimum Mn content in infant formulas in the US [1] may need to be updated to make it closer to the Mn content in breast milk, and a maximum Mn content for infant formulas and young child nutritional beverage products may be appropriate for the US market.

In the meantime, it must be noted that supplementation of infant formulas and young child nutritional beverage products with Mn is unnecessary to meet the current health-based regulatory minimum of 1 μg Mn/100 kcal stipulated by the CAC, EU and Republic of France, or the US minimum of 5 μg Mn/100 kcal [1–3,9,90]. There is no proven or likely benefit from Mn supplementation in these products and some researchers contend there is potential for adverse neurodevelopmental health effects in infants and young children. Pending research on dietary Mn exposures that examine health effects at high and low Mn dietary intakes in neonates, infants, and young children, formula manufacturers may consider taking measures to reduce Mn content in their products to approach the Mn concentrations found in breast milk rather than supplement their products with additional Mn when the products already have much higher Mn concentrations than breast milk.

Supporting information

S1 Table. Product label information.
(DOCX)

S2 Table. Reconstitution according to labels only.
(DOCX)

S3 Table. Labelled energy content.
(DOCX)

S4 Table. Labeled Mn content.
(DOCX)

S5 Table. Laboratory reconstitution measurements of powdered samples.
(DOCX)

S6 Table. Water and powder in 1 L of prepared formula.
(DOCX)

S7 Table. Mass of solids in 1 L of liquid samples (by evaporation starting with measured volumes).
(DOCX)

S8 Table. Calculations to derive g powder (or solids) / 100 kCal based on laboratory measurements of g powder (or solids) / L prepared formula and labeled energy content.
(DOCX)

S9 Table. PIXE data.
(DOCX)

S10 Table. Mean NIST measurements.
(DOCX)

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References

1. Code of Federal Regulations (21 CFR 107). Title 21—Food and drugs. Part 107—Infant formula. 2017. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=107. Cited 17 November 2017.
2. Codex Alimentarius Commission (CAC). Standard for infant formula and formulas for special medical purposes intended for infants. CODEX STAN 72–1981. 2016. Available from: http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/. Cited 9 November 2017.
3. ECEC0771649A. Journal Officiel de la République Française (Official Journal of the French Republic). Arrêté du 11 avril 2008 relatif aux préparations pour nourrissons et aux préparations de suite et modifiant l'arrêté du 20 septembre 2000 relatif aux aliments diététiques destinés à des fins médicales spéciales (Order of 11 April 2008 on infant formulas and follow-on formulas and amending the order of 20 September 2000 on dietary foods for special medical purposes). n˚0096 du 23 avril 2008 page 6700, texte n˚18, Version consolidée au 15 janvier 2018 (n˚0096 of April 23, 2008 page 6700, text n˚18, consolidated version on January 15, 2018). Available from: https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000018685743&dateTexte=20180115. Cited 15 January 2018.
4. Roels HA, Bowler RM, Kim Y, Henn BC, Mergler D, Hoet P, et al. Manganese exposure and cognitive deficits: A growing concern for manganese neurotoxicity. Neurotoxicology. 2012; 33(4):872–880. https://doi.org/10.1016/j.neuro.2012.03.009 PMID: 22498092
5. Grandjean P, Landrigan P Neurobehavioural effects of developmental toxicity. Lancet Neurol. 2014; 13: 330–38. https://doi.org/10.1016/S1474-4422(13)70278-3 PMID: 24556010
6. Lucchini R, Placidi D, Cagna G, Fedrighi C, Oppini M, Peli M, et al. Manganese and developmental neurotoxicity. Adv Neurobiol. 2017; 18:13–34. https://doi.org/10.1007/978-3-319-60189-2_2 PMID: 28889261
7. Neal AP, Guilarte TR. Mechanisms of lead and manganese neurotoxicity. Toxicol Res (Camb). 2013; 2(2):99–114. https://doi.org/10.1039/C2TX20064C PMID: 25722848
8. Valcke M, Bourgault M-H, Haddad S, Bouchard M, Gauvin D, Levallois P. Deriving a drinking water guideline for a non-carcinogenic contaminant: The case of manganese. Int J of Environ Res and Pub Health 2018; 15:1293. https://doi.org/10.3390/ijerph15061293 PMID: 29925794
9. European Commission (EC). Official Journal of the European Union. Commission delegated regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. 2015. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016R0127. Cited 1 February 2016.
10. Rodriguez-Barranco M, Lacasaña M, Aguilar-Garduño C, Alguacil J, Gil F, González-Alzaga B, Rojas-García A. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: A systematic review and meta-analysis. Sci Total Environ. 2013; 454–455:562–577. https://doi.org/10.1016/j.scitotenv.2013.03.047 PMID: 23570911
11. O'Neal SL, Zheng W. Manganese Toxicity Upon Overexposure: a Decade in Review. Curr Envir Health Rpt. 2015; 2:315–328. https://doi.org/10.1007/s40572-015-0056-x PMID: 26231508
12. Menezes-Filho JA, Bouchard M, Sarcinelli P, Moreira JC. Manganese exposure and the neuro psychological effect on children and adolescents: a review. Rev Panam Salud Public. 2009; 26:541–8. https://doi.org/10.1590/s1020-49892009001200010 PMID: 20107709
13. Bhang SY, Cho SC, Kim JW, Hong YC, Shin MS, Yoo HJ, et al. Relationship between blood manganese levels and children's attention, cognition, behavior, and academic performance—a nationwide cross-sectional study. Environ Res. 2013; 126:9–16. https://doi.org/10.1016/j.envres.2013.05.006 PMID: 23790803
14. Claus Henn B, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, et al. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted Superfund site. Environ Health Perspect. 2017; 125 (6):067020. https://doi.org/10.1289/EHP925 PMID: 28665786
15. Lee JJ, Valeri L, Kapur K, Ibne Hasan MOS, Quamruzzaman Q, Wright RO, et al. Growth parameters at birth mediate the relationship between prenatal manganese exposure and cognitive test scores among a cohort of 2- to 3-year-old Bangladeshi children. Int J Epidemiol. 2018; 47(4):1169–1179. https://doi.org/10.1093/ije/dyy069 PMID: 29733356
16. Mora AM, Córdoba L, Cano JC, Hernandez-Bonilla D, Pardo L, Schnaas L, et al. Prenatal Mancozeb Exposure, Excess Manganese, and Neurodevelopment at 1 Year of Age in the Infants' Environmental Health (ISA) Study. Environ Health Perspect. 2018; 126(5):057007. https://doi.org/10.1289/EHP1955 PMID: 29847083

17. Muñoz Rocha TV, Tamayo y Ortiz M, Romero M, Pantic I, Schnaas L, Bellinger D, et al. Prenatal co-exposure to manganese and depression and 24-months neurodevelopment. Neurotoxicology. 2018; 64:134–141. https://doi.org/10.1016/j.neuro.2017.07.007 PMID: 28728787

18. Nascimento SN, Barth A, Göethel G, Baierle M, Charão MF, Brucker N, et al. Cognitive deficits and ALA-D-inhibition in children exposed to multiple metals. Environ Res. 2015; 136:387–95. https://doi.org/10.1016/j.envres.2014.10.003 PMID: 25460660

19. Valeri L, Mazumdar MM, Bobb JF, Claus Henn B, Rodrigues E, Sharif OIA, et al. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20–40 months of age: evidence from rural Bangladesh. Environ Health Perspect. 2017; 125(6):067015. https://doi.org/10.1289/EHP614 PMID: 28669934

20. Yu XD, Zhang J, Yan CH, Shen XM. Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development. Environ Res. 2014; 133:232–8. https://doi.org/10.1016/j.envres.2014.04.012 PMID: 24971720

21. Yu X, Chen L, Wang C, Yang X, Gao Y, Tian Y. The role of cord blood BDNF in infant cognitive impairment induced by low-level prenatal manganese exposure: LW birth cohort, China. Chemosphere. 2016; 163:446–451. https://doi.org/10.1016/j.chemosphere.2016.07.095 PMID: 27565312

22. Kullar SS, Shao K, Surette C, Foucher D, Mergler D, Cormier P, et al. A benchmark concentration analysis for manganese in drinking water and IQ deficits in children. Environ Int. 2019; 130:104889. https://doi.org/10.1016/j.envint.2019.05.083 PMID: 31200154

23. Bjørklund G, Chartrand MS, Aaseth J. Manganese exposure and neurotoxic effects in children. Environ Res. 2017; 155:380–384. https://doi.org/10.1016/j.envres.2017.03.003 PMID: 28282629

24. Peres TV, Schettinger MRC, Chen P, Carvalho F, Avila DS, Bowman AB. Manganese-induced neurotoxicity: a review of its behavioral consequences and neuroprotective strategies. BMC Pharm Tox. 2016; 17:57. https://doi.org/10.1186/s40360-016-0099-0 PMID: 27814772

25. Zoni S, Lucchini RG. Manganese exposure: cognitive, motor and behavioral effects on children: a review of recent findings. Curr Opin Pediatr. 2013; 25:255–260. https://doi.org/10.1097/MOP.0b013e32835e906b PMID: 23486422

26. Betancourt Ó, Tapia M, Méndez I. Decline of general intelligence in children exposed to manganese from mining contamination in Puyango River Basin, Southern Ecuador. Ecohealth. 2015; 12(3):453–60. https://doi.org/10.1007/s10393-015-1027-2 PMID: 25851196

27. Bouchard MF, Surette C, Cormier P, Foucher D. Low level exposure to manganese from drinking water and cognition in school-age children. Neurotoxicology. 2018; 64:110–117. https://doi.org/10.1016/j.neuro.2017.07.024 PMID: 28716743

28. Dion LA, Saint-Amour D, Sauvé S, Barbeau B, Mergler D, Bouchard MF. Changes in water manganese levels and longitudinal assessment of intellectual function in children exposed through drinking water. Neurotoxicology. 2018; 64:118–125. https://doi.org/10.1016/j.neuro.2017.08.015 PMID: 28870865

29. Haynes EN, Sucharew H, Hilbert TJ, Kuhnell P, Spencer A, Newman NC, et al. Impact of air manganese on child neurodevelopment in East Liverpool, Ohio. Neurotoxicology. 2018; 64:94–102. https://doi.org/10.1016/j.neuro.2017.09.001 PMID: 28888663

30. Menezes-Filho JA, Carvalho CF, Rodrigues JLG, Araújo CFS, Dos Santos NR, Lima CS, et al. Environmental co-exposure to lead and manganese and intellectual deficit in school-aged children. Int J Environ Res Public Health. 2018; 15(11). https://doi.org/10.3390/ijerph15112418 PMID: 30384464

31. Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, Kline, et al. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect. 2006; 114:124–129. https://doi.org/10.1289/ehp.8030 PMID: 16393669

32. Bauer JA, Claus Henn B, Austin C, Zoni S, Fedrighi C, Cagna G, et al. Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility. Environ Int. 2017; 108:299–308. https://doi.org/10.1016/j.envint.2017.08.013 PMID: 28941415

33. Carvalho CF, Oulhote Y, Martorelli M, Carvalho CO, Menezes-Filho JA, Argollo N, et al. Environmental manganese exposure and associations with memory, executive functions, and hyperactivity in Brazilian children. Neurotoxicology. 2018; 69:253–259. https://doi.org/10.1016/j.neuro.2018.02.002 PMID: 29432852

34. García-Chimalpopoca Z, Hernández-Bonilla D, Cortez-Lugo M, Escamilla-Núñez C, Schilmann A, Riojas-Rodríguez H, et al. Verbal Memory and Learning in Schoolchildren Exposed to Manganese in Mexico. Neurotox Res. 2019. https://doi.org/10.1007/s12640-019-00037-7 PMID: 31148117

35. Hernández-Bonilla D, Schilmann A, Montes S, Rodríguez-Agudelo Y, Rodríguez-Dozal S, Solís-Vivanco R, et al. Effects of manganese exposure on visuoperception and visual memory in schoolchildren. Neurotoxicology. 2016; 57:230–240. https://doi.org/10.1016/j.neuro.2016.10.006 PMID: 27737811

36. Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology. 2003; 24(4–5):667–74. https://doi.org/10.1016/S0161-813X(03)00058-5 PMID: 12900080

37. Woolf A, Wright R, Amarasiriwardena C, Bellinger D. A child with chronic manganese exposure from drinking water. Environ Health Perspect. 2002; 110(6):613–6. https://doi.org/10.1289/ehp.02110613 PMID: 12055054

38. Claus Henn B, Austin C, Coull BA, Schnaas L, Gennings C, Horton MK, et al. Uncovering neurodevelopmental windows of susceptibility to manganese exposure using dentine microspatial analyses. Environ Res. 2018; 161:588–598. https://doi.org/10.1016/j.envres.2017.12.003 PMID: 29247915

39. Chiu YM, Claus Henn B, Hsu HL, Pendo MP, Coull BA, Austin C, et al. Sex differences in sensitivity to prenatal and early childhood manganese exposure on neuromotor function in adolescents. Environ Res. 2017; 159:458–465. https://doi.org/10.1016/j.envres.2017.08.035 PMID: 28858760

40. Dion LA, Bouchard MF, Sauvé S, Barbeau B, Tucholka A, Major P, et al. MRI pallidal signal in children exposed to manganese in drinking water. Neurotoxicology. 2016 Mar; 53:124–131. https://doi.org/10.1016/j.neuro.2016.01.004 PMID: 26801245

41. Lao Y, Dion LA, Gilbert G, Bouchard MF, Rocha G, Wang Y, et al. Mapping the basal ganglia alterations in children chronically exposed to manganese. Sci Rep. 2017; 7:41804. https://doi.org/10.1038/srep41804 PMID: 28155922

42. Aschner JL, Anderson A, Slaughter JC, Aschner M, Steele S, Beller A, et al. Neuroimaging identifies increased manganese deposition in infants receiving parenteral nutrition. Am J Clin Nutr. 2015; 102 (6):1482–9. https://doi.org/10.3945/ajcn.115.116285 PMID: 26561627

43. de Water E, Proal E, Wang V, Medina SM, Schnaas L, Téllez-Rojo MM, et al. Prenatal manganese exposure and intrinsic functional connectivity of emotional brain areas in children. Neurotoxicology. 2018; 64:85–93. https://doi.org/10.1016/j.neuro.2017.06.006 PMID: 28610744

44. Shih J-H, Zeng B-Y, Lin P-Y, Chen T-Y, Chen Y-W, Wu C-K, et al. Association between peripheral manganese levels and attention-deficit/hyperactivity disorder: a preliminary meta-analysis. Neuropsych Dis Treat. 2018; 14:1831–1842. https://doi.org/10.2147/NDT.S16537

45. Haynes EN, Chen A, Ryan P, Succop P, Wright J, Dietrich KN. Exposure to airborne metals and particulate matter and risk for youth adjudicated for criminal activity. Environ Res. 2011; 111(8):1243–8. https://doi.org/10.1016/j.envres.2011.08.008 PMID: 21864838

46. Horton MK, Hsu L, Claus Henn B, Margolis A, Austin C, Svensson K, et al. Dentine biomarkers of prenatal and early childhood exposure to manganese, zinc and lead and childhood behavior. Environ Int. 2018; 121(Pt 1):148–158. https://doi.org/10.1016/j.envint.2018.08.045 PMID: 30205321

47. Rodrigues JLG, Araújo CFS, Dos Santos NR, Bandeira MJ, Anjos ALS, Carvalho CF, et al. Airborne manganese exposure and neurobehavior in school-aged children living near a ferro-manganese alloy plant. Environ Res. 2018; 167:66–77. https://doi.org/10.1016/j.envres.2018.07.007 PMID: 30007874

48. Lucchini RG, Guazzetti S, Zoni S, Donna F, Peter S, Zacco A, Salmistraro M, et al. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. Neurotoxicology. 2012; 33(4):687–96. https://doi.org/10.1016/j.neuro.2012.01.005 PMID: 22322213

49. Lucchini RG, Zoni S, Guazzetti S, Bontempi E, Micheletti S, Broberg K. Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents. Environ Res. 2012; 118:65–71. https://doi.org/10.1016/j.envres.2012.08.003 PMID: 22925625

50. Parvez F, Wasserman GA, Factor-Litvak P, Liu X, Slavkovich V, Siddique AB, et al. Arsenic Exposure and Motor Function among Children in Bangladesh. Environ Health Persp. 2011; 119:1665–1670. https://doi.org/10.1289/ehp.1103548 PMID: 21742576

51. Rahbar MH, Samms-Vaughan M, Dickerson AS, Loveland KA, Ardjomand-Hessabi M, Bressler J, et al. Blood manganese concentrations in Jamaican children with and without autism spectrum disorders. Environ Health. 2014; 13:69. https://doi.org/10.1186/1476-069X-13-69 PMID: 25149876

52. Rink SM, Ardoino G, Queirolo EI, Cicariello D, Mañay N, Kordas K. Associations between hair manganese levels and cognitive, language, and motor development in preschool children from Montevideo, Uruguay. Arch Environ Occupat Health. 2013; 69:46–54. https://doi.org/10.1080/19338244.2012.7525229

53. Leonhard MJ, Chang ET, Loccisano AE, Garry MR. A systematic literature review of epidemiologic studies of developmental manganese exposure and neurodevelopmental outcomes. Toxicology. 2019; 420:46–65. https://doi.org/10.1016/j.tox.2019.03.004 PMID: 30928475

54. European Food Safety Authority (EFSA). Scientific opinion on the essential composition of infant and follow-on formulae. EFSA J. 2014; 12(7):3760. https://doi.org/10.2903/j.efsa.2014.3760.

55. Casey CE, Smith A, Zhang P. Microminerals in Human and Animal Milks. In Jensen RG (editor). Handbook of Milk Composition. 1995. San Diego: Academic Press, pp. 622–674.

56. Ljung K, Palm B, Grandér M, Vahter M. High concentrations of essential and toxic elements in infant formula and infant foods–A matter of concern. Food Chem. 2011; 127:943−951. https://doi.org/10.1016/j.foodchem.2011.01.062 PMID: 25214082

57. Stastny D, Vogel RS, Picciano MF. Manganese intake and serum manganese concentration of human milk-fed and formula-fed infants. Am J Clin Nutr. 1984; 39(6):872–8. https://doi.org/10.1093/ajcn/39.6.872 PMID: 6539060

58. Pandelova M, Levy Lopez W, Michalke B, Schramm KW Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. J Food Comp Anal. 2012; 27(2): 120–127. https://doi.org/10.1016/j.jfca.2012.04.011

59. Dörner K, Dziadzka S, Hohn A, Schaub J. Longitudinal manganese and copper balances in young infants and preterm infants fed on breast-milk and adapted cow's milk formulas. Br J Nutr. 1989; 61:559−572. https://doi.org/10.1079/bjn19890143 PMID: 2758010

60. Paquette LH, and Thompson JJ. Minerals and trace elements in milk, milk products, infant formula, and adult/pediatric nutritional formula, ICP-MS method: Collaborative study, AOAC final action 2015.06, ISO/DIS 21424, IDF 243. Journal of AOAC International 2018; 101(2):536–561. https://doi.org/10.5740/jaoacint.17-0318 PMID: 29151407

61. Poitevin E. Official methods for the determination of minerals and trace elements in infant formula and milk products: A review. Journal of AOAC International. 2016; 99(1):42–52. https://doi.org/10.5740/jaoacint.15-0246 PMID: 26821839

62. Aras NK, Ataman OY. X-ray methods. In: Aras NK, Ataman OY, editors. Trace element analysis of food and diet. Cambridge, UK: The Royal Society of Chemistry; 2006. p. 193−204. https://doi.org/10.1039/9781847552495

63. Akanle OA, Balogun FA, Owa JA, Spyrou NM. Study of the nutritional status of maternal breast milk in preterm infants in Nigeria. J. Radioanal. Nucl. Chem. 2000; 244:231−235. https://doi.org/10.1023/A:1006713626499

64. Balogun FA, Akanle OA, Spyrou NM, Owa JA. A comparative study of elemental composition of human breast milk and infant milk substitutes. Biol Trace Elem Res. 1994;Fall, 43−45;471−479. https://doi.org/10.1007/bf02917349 PMID: 7710863

65. Castro Gonzalez NP, Moreno-Rojas R, Calderón Sánchez F, Moreno Ortega A, Juarez Meneses M. Assessment risk to children's health due to consumption of cow's milk in polluted areas in Puebla and Tlaxcala, Mexico. Food Addit Contam Part B. 2017; 10(3):200−207. https://doi.org/10.1080/19393210.2017.1316320 PMID: 28393675

66. Khatun R, Ahasan MM, Abedin MJ, Akter S. Study of human milk in terms of sampling time and age of the lactating mothers. SUST J Sc Tech. 2012; 20(6):80−83.

67. Olabanji SO, Buoso MC, Ceccato D, Haque AMI, Cherubini R, Moschini G. PIGE-PIXE analysis of human milk. Nucl Instrum Methods Phys Res B. 1996; 109/110:258−261. https://doi.org/10.1016/0168-583X(95)00918-3

68. Solis C, Isaac-Olive K, Mireles A, Vidal-Hernandez M. Determination of trace metals in cow's milk from waste water irrigated areas in Central Mexico by chemical treatment coupled to PIXE. Microchem J. 2009; 91(1):9−12. https://doi.org/10.1016/j.microc.2008.06.001

69. Spyrou NM. Variations in trace element concentrations in breast milk with stages of lactation. J Radioanal Nucl Chem. 2001; 249(1):71–75.

70. Crinella FM. Does soy-based infant formula cause ADHD? Update and public policy considerations. Expert Rev Neurother. 2012; 12(4):395–407. https://doi.org/10.1586/ern.12.2 PMID: 22449212

71. Teddlie C, Yu F. Mixed methods sampling, A typology with examples. J Mix Methods Res. 2007; 1 (1):77–100. https://doi.org/10.1177/2345678906292430.

72. Sorieul S, Alfaurt P, Daudin L, Serani L, Moretto P. AIFIRA: An ion beam facility for multidisciplinary research. Nucl Instrum Methods Phys Res B. 2014; 332:68−73. https://doi.org/10.1016/j.nimb.2014.02.032

73. Johansson SAE, Johansson T. Analytical application of particle induced X-ray emission. Nucl Instr Meth Phys Res. 1976; 137(3): 473−516. https://doi.org/10.1016/0029-554X(76)90470-5

74. Carmona A, Devès G, Ortega R. Quantitative micro-analysis of metal ions in subcellular compartments of cultured dopaminergic cells by combination of three ion beam techniques. Anal Bioanal Chem. 2008; 390 (6):1585−1594. https://doi.org/10.1007/s00216-008-1866-6 PMID: 18246461

75. Perrin L., Carmona A., Roudeau S., Ortega R. Evaluation of sample preparation methods for single cell quantitative element imaging using proton or synchrotron radiation focused beams. J Anal Atom Spectrom. 2015; 30: 2525–2532. https://doi.org/10.1039/C5JA00303B

76. Chu WK, Mayer JW, Nicolet MA. Backscattering spectrometry. Orlando, FL: Academic Press, Inc., 1978.

77. Ortega R, Devès G, Carmona A. Bio-metals imaging and speciation in cells using proton and synchrotron radiation X-ray microspectroscopy. J R Soc Interface. 2009; 6:S649–S658. https://doi.org/10.1098/rsif.2009.0166.focus PMID: 19605403

78. Campbell JL, Boyd NI, Grassi N, Bonnick P, Maxwell JA. The Guelph PIXE software package IV. Nucl Instrum Methods Phys Res B. 2010; 268(20):3356–3363. https://doi.org/10.1016/j.nimb.2010.07.012

79. Mayer M. SIMNRA, a simulation program for the analysis of NRA, RBS and ERDA. In: Duggan JL, Morgan IL, editors. Proceedings of the 15th international conference on the application of accelerators in research and industry. College Park, MD: American Institute of Physics Conference Proceedings; 1999;475:541.

80. Code of Federal Regulations (21 CFR 101.9). Title 21—Food and drugs. Part 101—Food labeling. Subpart A—General Provisions. §101.9—Nutrition labeling of food. 2017. Available from: https://www.ecfr.gov/cgi-bin/text-idx?SID=13b6ba5fb985cc64c070a3bc1f5e6a44&mc=true&node=se21.2.101_19&rgn=div8. Cited 28 November 2017.

81. Pacquette LH, Thompson JJ. Minerals and trace elements in milk, milk products, infant formula, and adult/pediatric nutritional formula, ICP-MS Method: Collaborative study, AOAC Final Action 2015.06, ISO/DIS 21424, IDF 243. J AOAC Int 2018; 101(2):536–561. https://doi.org/10.5740/jaoacint.17-0318 PMID: 29151407

82. Skoog DA, West DM, Holler FJ, Crouch SR. Fundamentals of analytical chemistry. 9th ed. Boston: Brooks/Cole Division of Thomson Learning, Inc., 2014. p. 86.

83. Collipp PJ, Chen SY. Maitinsky S. Manganese in infant formulas and learning disability. Ann Nutr Metab. 1983; 27:488–494. https://doi.org/10.1159/000176724 PMID: 6651226

84. Arnaud J, Favier A. Copper, iron, manganese and zinc contents in human colostrum and transitory milk of French women. Sci Total Environ. 1995; 159:9–15. https://doi.org/10.1016/0048-9697(94)04314-d PMID: 7846513

85. Fuente MA de la, Olano A, Juârez M. Distribution of calcium, magnesium, phosphorus, zinc, manganese, copper and iron between the soluble and colloidal phases of ewe's and goat's milk. Lait. 1997; 77:515–520. https://doi.org/10.1051/lait:1997437

86. Krachler M, Prohaska T, Koellensperger G, Rossipal E, Stingeder G. Concentrations of selected trace elements in human milk and in infant formulas determined by magnetic sector field inductively coupled plasma–mass spectrometry. Biol Trace Elem Res. 2000; 76:97–112. https://doi.org/10.1385/BTER:76:2:97 PMID: 11049226

87. Leotsinidis M, Alexopoulos A, Kostopoulou-Farri E. Toxic and essential trace elements in human milk from Greek lactating women: Association with dietary habits and other factors. Chemosphere. 2005; 61:238–247. https://doi.org/10.1016/j.chemosphere.2005.01.084 PMID: 16168747

88. Codex Alimentarius Commission (CAC). Standard for infant formula and formulas for special medical purposes intended for infants. CODEX STAN 72–1981. 2007. Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/fr/?lnk=1&url= https://workspace.fao.org/sites/codex/Standards/CODEX+STAN+72-1981/CXS_072e.pdf. Cited 17 March 2018.

89. Codex Alimentarius Commission (CAC). Standard for follow-up formula. CODEX STAN 156–1987. 2017. Available from: http://www.fao.org/input/download/standards/293/CXS_156e.pdf. Cited 17 March 2018.

90. European Commission (EC). Official Journal of the European Union. Commission directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending. 2006. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32006L0141. Cited 17 February 2018.

91. European Food Safety Authority (EFSA). Scientific opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. 2013. Available from: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3408/epdf. Cited 13 March 2018.

92. Koletzko B, Baker S, Cleghorn G, Neto UF, Gopalan S, Hernell O, et al. Global standard for the composition of infant formula: Recommendations of an ESPGHAN coordinated international expert group. J Pediatr Gastroenterol Nutr. 2005; 41:584–599. https://doi.org/10.1097/01.mpg.0000187817.38836.42 PMID: 16254515

93. Lönnerdal B. Nutritional aspects of soy formula. Acta Paediatr Suppl. 1994; 402:105–108. PMID: 7841612

94. Cockell KA, Bonacci G, Belonje B. Manganese content of soy or rice beverages is high in comparison to infant formulas. J Am Coll Nutr. 2004; 23(2):124–130. https://doi.org/10.1080/07315724.2004.10719352 PMID: 15047678

95. Gamela RR, Duarte AT, Barrera EG, Welz B, Dessuy MB, Silva MM da, et al. Development of analytical methods for the determination of copper and manganese in infant formula using high resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis. Anal Methods. 2017; 9:2321−2327. https://doi.org/10.1039/C6AY03332F

96. McKinstry PJ, Indyk HE, Kim ND. The determination of major and minor elements in milk and infant formula by slurry nebulisation and inductively coupled plasma—optical emission spectrometry (ICP-OES). Food Chem. 1999; 65:245–252. https://doi.org/10.1016/S0308-8146(98)00183-6

97. Thompkinson DK, Kharb S. Aspects of infant food formulation. Compr Rev Food Sci F. 2007; 6:79 −102. https://doi.org/10.1111/j.1541-4337.2007.00020.x

98. Pennington JAT, Schoen SA, Salmon GD, Young B, Johnson RD, Marts RW. Composition of core foods of the U.S. food supply, 1982–1991: III. Copper, manganese, selenium, and iodine. J Food Compos Anal. 1995; 8(2):171–217. https://doi.org/10.1006/jfca.1995.1014

99. Yang X, Ye ZQ, Shi CH, Zhu ML, Graham RD. Genotypic differences in concentrations of iron, manganese, copper, and zinc in polished rice grains. J Plant Nutr. 1998; 21(7):1453–1462. https://doi.org/10.1080/01904169809365495

100. Amorim FR de, Nascentes CC, Franco MB, Silva JBB da. Fast determination of manganese in milk and similar infant food samples using multivariate optimization and GF AAS. Int J Spectrosc. 2011;810641. https://doi.org/10.1155/2011/810641

101. Bagdat S, Baran EK, Tokay F. Element fractionation analysis for infant formula and food additives by inductively coupled plasma optical emission spectrometry. Int J Food Sci Tech. 2014; 49;392−398.

102. Concha G, Eneroth H, Hallström H, Sand S. Contaminants and minerals in foods for infants and young children. Part 2: Risk and benefit assessment. 2013. https://www.livsmedelsverket.se/globalassets/rapporter/2013/2103_livsmedelsverket_1_part_2_contaminants_and_minerals_in_foods_for_infants_and_young_children_risk_and_benefit_assessment.pdf. Cited 19 October 2017.

103. Pashkova GV. X-ray fluorescence determination of element contents in milk and dairy products. Food Anal Methods. 2009; 2:303−310. https://doi.org/10.1007/s12161-009-9080-5

104. Peixoto RRA, Oliveira A, Cadore S. Multielemental determinations in chocolate drink powder using multivariate optimization and ICP OES. J Agric Food Chem. 2012; 60:8117−8122. https://doi.org/10.1021/jf303022r PMID: 22849827

105. Rehman S, Husnain SM. Assessment of trace metal contents in chocolate samples by atomic absorption spectrometry. J Trace Elem Anal. 2012; 1(1):1−11. https://doi.org/10.7726/jtea.2012.1001

106. Sager M. Chocolate and cocoa products as a source of essential elements in nutrition. J Nutr Food Sci. 2012; 2:123. https://doi.org/10.4172/2155-9600.1000123

107. Yanus RL, Sela H, Borojovich EJC, Zakon Y, Saphier M, Nikolski A, et al. Trace elements in cocoa solids and chocolate: An ICPMS study. Talanta. 2014; 119:1−4. https://doi.org/10.1016/j.talanta.2013.10.048 PMID: 24401377

108. Ahmad I, Zaman A, Samad N, Ayaz MM, Rukh S, Akbar A, et al. Atomic absorption spectrophotometery detection of heavy metals in milk of camel, cattle, buffalo and goat from various areas of Khyber-Pakhtunkhwa (KPK), Pakistan. J Anal Bioanal Tech. 2017; 8:367. https://doi.org/10.4172/2155-9872.1000367

109. Coni E, Bocca A, Coppolelli P, Caroli S, Cavallucci C, Marinucci MT. Minor and trace element content in sheep and goat milk and dairy products. Food Chem. 1996; 57(2):253−260.

110. Elhardallou SB, El-Naggar AY. Determination of micro minerals in milk from farm and pasture-reared cow, goat and camel; using inductively coupled plasma-optical emission spectrometry. Orient J Chem. 2016; 32(1):341−347. https://doi.org/10.13005/ojc/320138

111. Code of Federal Regulations (21 CFR 106.3). Title 21—Food and drugs. Part 106—Infant formula requirements pertaining to current good manufacturing practice, quality control procedures, quality factors, records and reports, and notifications. Subpart A- General provisions. §106.3 Definitions. 2018. Available from: https://www.ecfr.gov/cgi-bin/text-idx?SID=3a1a2e85dc99eefc557fa981e1c5a37a&mc=true&node=pt21.2.106&rgn=div5#se21.2.106_13. Cited 12 January 2018.

112. Code of Federal Regulations (21 CFR 105.3). Title 21—Food and drugs. Part 105—Foods for special dietary use. Subpart A—General provisions. §105.3 Definitions and interpretations. 2018. Available from: https://www.ecfr.gov/cgi-bin/text-idx?SID=b27c3cb20fafd344e33494b3d7897d6e&mc=true&node=pt21.2.105&rgn=div5#se21.2.105_13. Cited 12 January 2018.

113. Palafox MJR, Harris JL. Toddler formulas: Nutritional value and marketing claims. FASEB J. 2017; 31 (1):Supplement 169.5.

114. Pomeranz JL, Romo Palafox MJ, Harris JL. Toddler drinks, formulas, and milks: Labeling practices and policy implications. Prev Med 2018; 109:11–16. https://doi.org/10.1016/j.ypmed.2018.01.009 PMID: 29339115

115. Lönnerdal B. Hernell O. An opinion on "staging" of infant formula: A developmental perspective on infant feeding. J Pediatr Gastroenterol Nutr. 2016; 62(1):9–21. https://doi.org/10.1097/MPG.0000000000000806 PMID: 25844707

116. Klein LD, Breakey AA, Scelza B, Valeggia C, Jasienska G, Hinde K. Concentrations of trace elements in human milk: Comparisons among women in Argentina, Namibia, Poland, and the United States. PLoS One. 2017; 12(8):e0183367. https://doi.org/10.1371/journal.pone.0183367 PMID: 28817665

117. Berry NJ, Jones SC, Iverson DC. Circumventing the WHO Code? An observational study. Arch Dis Child. 2012; 97(4):320–325. https://doi.org/10.1136/adc.2010.202051 PMID: 21719442

118. World Health Organization (WHO). Cross-promotion of infant formula and toddler milks. Available from https://www.who.int/nutrition/publications/infantfeeding/information-note-cross-promotion-infant-formula/en/. Cited 4 June, 2019.

119. Agence française de sécurité sanitaire des aliments (AFSSA; French Food Safety Agency). Avis de l'Agence française de sécurité sanitaire des aliments relatif à l'évaluation d'un aliment diététique destiné aux nourrissons, enrichi en manganèse (Opinion of the French Food Safety Agency concerning the evaluation of a dietary food for infants, enriched in manganese). 2002. Available from: https://www.anses.fr/fr/system/files/NUT2001sa0090.pdf. Cited 17 March 2018.

120. Code of Federal Regulations (21 CFR 184). Title 21—Food and drugs. Part 184—Direct food substances affirmed as generally recognized as safe. Subpart B—Listing of specific substances affirmed as GRAS. §184—Manganese. 1985. Available from: https://www.ecfr.gov/cgi-bin/text-idx?SID=4cfb299566660110dba593fb82b371e1&mc=true&node=pt21.3.184&rgn=div5#se21.3.184_11446. Cited 19 November 2017.

121. United States Food and Drug Administration (US FDA). SCOGS (Select Committee on GRAS Substances). 2017. Available from: http://www.accessdata.fda.gov/scripts/fdcc/?set=SCOGS. Cited 17 November 2017.