Selenium Content of Spanish Infant Formula and Human Milk: Influence of Protein Matrix, Interactions with Other Trace Elements and Estimation of Dietary Intake by Infants

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Summary

The Selenium content of infant formula varies as a result of differences in the amount of intrinsic selenium compounds. Manufacturers have been gradually changing the protein profile of infant formula to reflect human milk contents more closely. Because of these variations in infant formula composition and their potential impact on selenium content, this trace element was analysed with regard to the different protein sources.

The aims of this study were to determine the selenium content in infant formulae sold commercially in Spain, to estimate a daily dietary intake for infants fed on formulae and to compare with the selenium provided by Spanish breast milk samples used as a reference. We have also identified certain trace elements added to formulae which interact with selenium according to type and protein matrix of infant formula.

Selenium concentration was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) with a hydride generator.

The selenium concentrations in human milk and infant formula determined in this study are similar to those found by other researchers in different countries. Daily intake of studied formulae was estimated according to recommended doses from manufacturers. Theoretical intake of lactating infants has been studied in relation to the Recommended Dietary Allowance (RDA: 10 µg Se/day) and the specific recommendations for infant formula nutrient contents (10 – 35 µg/L) set by Expert Panel of Life Sciences Research Office (LSRO) of the American Society for Nutritional Sciences.

According to our results, on an overall view, infants fed on the studied infant formulae have an intake between the basal and normative requirements. This might be considered to provide an adequate selenium intake. However, several formulae included in this research could provide an intake of selenium that does not reach the RDA for the first month of neonate life.

Keywords: Selenium, Infant formula, Human milk, Daily Intake, Recommended Dietary Allowances.

Introduction

The growing infant's need for essential trace elements has been relatively well established. It has become obvious that the greatest health risks of selenium deprivation are to be found among infants. Low intakes of selenium have been associated with two diseases of childhood (Keshan disease and Kaschin-Beck disease) (1).

The selenium intake of infants is of interest because of their rapid growth rate and their reliance on infant formula; particularly if it is considered that formula may be used as a sole source of nourishment from birth, often for extended time periods, and consequently provides the only source of selenium (2).

Breast milk provides an adequate supply of all essential nutrients for the neonate and its composition is therefore used as a reference. This is due to the fact that infants fed on human milk do not generally show micronutrient deficiencies. Special attention must be paid to pre-term infants due to their lower selenium store at birth.

Selenium levels in human milk are variable depending on the mother's dietary intake, geographical area and stage of lactation (3). However, infants fed human milk containing over 10 μg/L are not considered to be at risk from selenium deficiency. Most formula fed infants have a significantly lower selenium status than breast fed infants, though no adverse effects have been reported (4).

Selenium content of infant formula varies as a result of differences in the amounts of intrinsic selenium compounds. Little information on the selenium content of different types of commercial infant formula, primarily derived from the protein source used, is available. The selenium level is intrinsic to the geographic origin of the raw material (cow's milk or soy) used in the manufacture, or may be even lower due to possible losses occurring during the manufacture operations.

Infant selenium requirements have often been calculated by indirect approaches. The first estimation was based on extrapolation from animal data. Balance study of healthy breast fed babies or comparison of selenium intakes in those areas with and without selenium deficiency disease, constitute a way of pinpointing the adequate infant selenium requirement. Finally, another approach derives from the biochemical indices of selenium status, determining experimentally the requirement for dietary selenium based on saturation of plasma glutathione peroxidase activity. According to this last method, selenium requirements are calculated as 5 μg/day for infants up to 6 years old.

This procedure was adopted by the Food and Nutrition Board (1989), extrapolating from adults on the basis of body weight. Therefore application of a safety factor to the above requirement would result in a minimum adequate selenium intake of 10 μg/day (Recommended Dietary Allowance, RDA) for infants (2).

On the other hand, Levander (5) reviews experimental data and evidence available for estimating a selenium intake for infants and he suggests standardising the selenium content of infant formula in order to provide a minimum of 10 μg and a maximum of 45 μg of selenium daily.

Recently, a Joint FAO/IAEA/WHO Expert Committee on trace elements in human nutrition, has reported two different concepts of trace element requirements, namely basal and normative requirements. Basal requirement refers to the intake needed to prevent pathologically relevant and clinically detectable signs of impaired functions attributable to inadequacy of the nutrient. Normative requirements are the intakes needed to maintain a level of tissue storage. These have been established as 3 and 6 μg/day for infants 0-3 months old, 5 and 9 μg/day for infants 3-6 months old, 6 and 12 μg/day for infants 6-12 months old, respectively (6).

International paediatric organisms have studied these micronutrients thoroughly, estimating both the adequate daily dietary intake and the safety range which would fulfil selenium needs in most healthy neonates (7, 8).

The Expert Panel of LSRO (American Society for Nutrition Sciences) has proposed the minimum (10 μg/L) and maximum (35 μg/L) selenium content to be necessarily found in infant formula serving as a sole source of nutrition for term infants throughout the first year of life (8). The LSRO staff have considered the estimated selenium concentration of human milk in countries in which selenium deficiency has not been recognised in breast fed infants and a conservative estimation for the lower bioavailability of selenium from soya formulae. The maximum value has been set to be similar to the upper limits of selenium found in human milk and sufficiently less than the intake associated with development of selenosis.

Until recently, selenium was not added as a specific nutrient to commercial infant formula. Nevertheless, caution is still exercised by manufacturers of infant formulae with selenium supplementation. In Europe, EEC guidelines 96/4/EC authorise the fortification of formulae with inorganic forms of selenium (selenite and selenate) (9).

There is some evidence that infant formulae are fortified. Although it has been demonstrated that biochemical signs of selenium status improve in selenium supplemented formula fed infants as compared to unsupplemented formula fed children, no observable clinical benefits have been demonstrated (10).

Protein changes made during recent years in the newer formulations for infants, the influence of geographical origin of the raw material and feeding with formula as the sole source of nutrition for extended periods, make it imperative to monitor the selenium content of infant formula in order to guarantee the neonate's adequate intake of selenium. The aims of this study were to determine the selenium content in infant formulae sold commercially in Spain, to estimate a daily dietary intake for infants fed on formulae and to compare with the selenium provided by Spanish breast milk samples used as a reference. At the same time, we try to discuss the implications and possible interactions of trace elements with selenium content depending on protein matrix of infant formula.

Material and Methods

Sampling

Infant formulae were purchased directly from a distribution company in Pamplona (Spain). 82 different infant formulae from 10 different manufacturers were studied. Formulae included both powder (n = 61) and ready to use (n = 14) preparations, such as soy-based formulae (n = 7) or those based on cow's milk (n = 68) (classified as follows: starter formula -adapted (n = 16) and non adapted (n = 4)-; follow-up formula (n = 19); specialised formula - hypoallergenic formulae (n = 12), designed for lactose intolerant (n = 7), or inborn errors of metabolism (n = 10)-;and, pre-term formula (n = 7)). Special care was taken to minimise the risk of adventitious contamination when handling. Infant formula containers were stored in the absence of light at room temperature in a humidity controlled room. Infant formula samples were opened in the clean laboratory under flow laminar bench.

Individual mature breast milk samples were taken from young healthy mothers living in Pamplona (Spain). A total of 31 human milk samples were used in this research. The human milk was obtained by exerting gentle manual expression of the breast, using vinyl talc free gloves (Rotiprotect®, Carl Roth, Karlsruhe, Germany) and plastic material (Plastibrand®, Brand, Wertheim, Germany) to carry out the sampling. After collection, fresh human milk samples were immediately kept frozen at –20°C in appropriate

polypropylene containers. Special care and handling rules were adopted to minimise every possible source of contamination from the sampling step onwards. All plastic material used in sample handling and analysis was cleaned previously in 5 % nitric acid solution (Merck, Darmstadt, Germany) for six days and later rinsed three times with ultrapure water before utilisation. Plastic instruments were kept under cover until use.

Sample treatment

Human milk (1.000 mL) and infant formula (0.3000 g powder, 1.5 mL liquid) samples were digested with 4 mL of sub-boiling nitric acid (Merck, Darmstadt, Germany) in a closed acid-decomposition system equipped with a high pressure Teflon digestion bombs and a microwave oven (Milestone MLS 1200, Millestone s.r.l. Sorisole, Italy). The samples were mineralised under the conditions summarised in table 1. When digestion was complete, 2 mL of suprapure hydrochloric acid (Merck, Darmstadt, Germany) was added in order to reduce Se (VI) to Se (IV). The mixture was heated at 90 ºC for 10 minutes. Solutions obtained were then made up to 10 mL with ultrapure deionized water and kept stored frozen at -20 ºC until analysis. Samples were digested in triplicate.

Analytical methods

The selenium concentration was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) with a hydride generator (Jobin Ybon JY 38S Plus Sequential, Instruments S.A., France). Operating parameters of the instrument are given in table 2.

The selenium calibration curve was calculated using direct calibration against aqueous standards. Working standard solutions (0 to 10 μg/L) were made up each day by dilution from stock 1000 mg/L standard solution (Merck, Darmstadt, Germany) in enough hydrochloric acid to a final acid concentration similar to prepared samples. The values reported are the mean of triplicate assays.

In each analytical batch, blank reagents, aqueous internal standard and reference material were included to provide on-going quality control. Background analytical levels of selenium were determined by running blank reagents. The levels obtained were insignificant. The methodological detection limit was established based on three times the blank assay standard deviation and corresponded to 1.4 μg/L for infant formula. Aqueous internal standard containing 2.0 μg/L was run throughout the course of the analysis. Fairly good agreement was obtained with our findings of 1.9 ± 0.2 μg/L (n = 34).

The IAEA (International Atomic Energy Agency) milk powder A 11 was used as internal standard to establish the quality control. Prior to analysis, standard A 11 (0.500 g) was tested with the same techniques as the samples in order to guarantee accuracy and precision. Our results (34.14 ± 7.97 ng/g, n = 12) were in good fit with the certified level (33.9 ng/g, ranged 26.7 – 41.1 ng/g). Additionally, the recovery of spiked selenium at different concentrations (1 – 5 μg/L) in infant formula was thought to be satisfactory (n = 6, 94.6 – 102.3 %). No evidence of selenium loss in the digestion step was found.

Zinc and copper concentrations in digested acid solutions were analysed by flame atomic absorption spectrophotometry (FAAS, GBC 902, Dandenong, Victoria, Australia). Aluminium levels were determined by electrothermal atomisation (ETAAS, GBC GF 2000, Dandenong, Victoria, Australia). Inductively coupled plasma atomic emission spectrometry (Jobin Ybon JY 38S Plus Sequential, Instruments S.A., France) with Meinhard nebulizer was used for manganese. Zinc, copper, aluminium and manganese calibration curves were accomplished using direct calibration against aqueous standards. Details of measurements, operating parameters and trace element quantification have been described elsewhere (11, 12).

Chemicals

Suprapure nitric acid was purchased from Merck (Merck, Darmstadt, Germany) and distilled by sub-boiling before use. Ultrapure deionized water type Milli Q was used for preparation and/or dilution of treated sample and standard solutions. Suprapure hydrochloric acid (Merck, Darmstadt, Germany) was added to convert the selenium to Se(IV) state. A solution of sodium borohydride 2 % (Merck, Darmstadt, Germany) stabilised by sodium hydroxide was used as reactive to determine selenium.

Statistical Analysis

Statistical analysis was performed with SPSS v.9.0 for Windows program.

Results and Discussion

Selenium contents

Table 3 shows the mean concentrations of analysed selenium in the different types of infant formulae and breast milk samples investigated. Selenium concentration found in

powdered infant formula was calculated according to the manufacturer's dilution instructions to express the content in μg/L.

Selenium concentration in most infant formulae investigated is lower than in the breast milk samples (16.3 ± 4.7 μg/L). It is well documented that its content varies widely due to dietary selenium intake of the mother and this is reflected in wide variations in human milk selenium content. The concentration value determined is very similar to the selenium values of human milk reported by other researchers in different countries (13-23) (table 4).

Likewise, the wide variability observed in selenium concentration in different commercial formulae studied is of special relevance. It was not possible to establish a relation of dependence based on selenium content in accordance with the type of infant formula following the standard classification for infant feeding.

Most researchers have also reported large variations in selenium content, even from one batch to another. The concentration range in this study is similar to those found by other authors in Europe and America, and lower than in infant formulae studied from Asia (15, 17, 18, 20, 23-35) (table 5).

Selenium content in infant formula is primarily associated with protein (3,4). Figure 1 shows large variations in selenium levels depending on the protein source used. There is a substantial difference between brands of infant formula, due possibly to the geographical differences in milk or soy used and the varied processing methods and conditions employed during the protein separation in its manufacture.

It has been known that the level of dietary selenium is normally associated with the protein fraction used in manufacture of infant formula, for example, whey, casein, hydrolysate or soya protein. At present, the tendency is to reduce the protein content, replace casein and enrich in whey protein to more closely reflect, as far as possible, the protein pattern found in human milk (20).

Table 6 summarises the average and ranges of selenium content classified according to the source protein contained and different types of researched formulae. Our results have confirmed the lower selenium content of whey-based (7.4 ± 2.8 μg/L) than casein-based (8.7 ± 1.8 μg/L) formulae for adapted starter formulae, although, as is the case in human milk, large variations in selenium content might occur depending on geochemical area of origin, industrial process and separation method of protein from the raw material (cow's milk).

Soya formulae tend to have the lowest levels of selenium (6.7 ± 2.6 μg/L), showing the lowest concentration range (1.5 – 9.8 μg/L). These selenium levels are in agreement with the low concentrations and the influence of geographic variation on the selenium content of soya beans (36).

However, preterm formulae provide the highest mean value of selenium found (15.6 ± 9.5 μg/L), ranging from 10.2 – 36.2 μg/L. Probably, these values occur in connection with the studies that deliberately promote selenium fortification for premature infants in order to avoid the potential risk of exposure to oxidative stress at birth (37, 38).

The large variations found in hypoallergenic specialised formulae are significant (14.4 ± 9.9 μg/L; range 5.7 – 37.2). This group includes two different types of formulae depending on protein hydrolysate content. Selenium contents in whey hydrolysed HA formulae (17.0 ± 11.9 μg/L) are higher than in casein hydrolysed HA formulae (10.8 ± 5.5 μg/L). Protein hydrolysis treatment provides selenium enrichment in these formulae and whey hydrolysate is a better source than casein hydrolysate (Table 2).

Selenium levels in follow-up formulae (11.6 ± 7.2 μg/L) are higher than those found in starter formulae (adapted, 9.2 ± 3.3 μg/L; non adapted 9.3 ± 6.0 μg/L), as a result of the mean protein source, whole or skimmed milk, used in the formula manufacture for infants aged more than 5 months (Table 6). It is clear that the milk-skimming process influences the selenium concentration. Whole-milk-based follow-up formulae provide the higher concentrations (15.9 ± 10.3 μg/L, n = 5), whereas skim-milk and whey-based formulae provide 10.5 ± 6.5 μg/L (n = 13) and 6.1 (n =1), respectively.

We could therefore conclude that the variability observed in the selenium content of the studied formulae is not related to different kinds of infant formula but rather it depends on the intrinsic selenium coming from the protein matrix used in the infant formula manufacture. Starter formulae observe the EEC legislation by which the maximum selenium concentration (22.5 μg/L) for infant formulae for infants up to 6 months old is established in Directive 96/4/EC.

Finally, in general, the infant formulae investigated are close to the proposed selenium contents (10 – 35 μg/L) recommended by the Expert Panel of Life Sciences Research Office (LSRO) of the American Society for Nutritional Sciences (8).

Manufacturer and obtained values

Manufacturers report the contents of several trace elements in the label information given on infant formula. But selenium values are not always included on this, and nowhere is information about supplemented selenium salt available. It is not clear, but selenium was probably not added by manufacturers to the infant formulae under study here.

In order to compare, table 7 includes the concentrations of selenium in infant formula types considering their state of aggregation with values indicated on the label by different commercial brands. No levels of statistical significance were found, although it is possible to point out that most formulae do not inform about selenium content and as is suspected, statistical tests have a limited value. They could always conclude that the concentration range is similar in a wide interval to certain labelled values.

Daily dietary selenium intake

As has been mentioned previously, selenium concentration values are found to be close to recommended levels. But this is not enough evidence to avoid the potential risk of deficiency. In this respect, the estimation of the theoretical intake in lactating infant formula, considering pre-term infant as a special case, seems to be advisable.

We have estimated the amount of selenium supplied by different commercial formulae and human milk samples, calculating a daily dietary selenium intake for infants fed on formulae and breast milk. A detailed comparison with Recommended Dietary Allowance (RDA) has proved to be very useful. Taking into consideration the different stages of infancy, as well as the fact that most infants observe similar feeding regimens, we have worked out a percentage of satisfaction compared with RDA. Daily intake of formulae has been estimated according to recommended doses, both in terms of weight and volume (for powder or ready to use formulae).We must also take into account the fact that most of the infants fed with follow-up formulae also receive beikost nutrients, which may increase the trace element intake as a result of the variety of foods introduced. The NRC advises a RDA for selenium of 10 µg/day up to sixth months and 15 µg/day from six months to one year of life, in infants who are fed with infant formulae (2).

Figures 2 and 3 show the estimated daily intake and the calculated satisfaction percentage of RDA for the newborn period to the first year of life. Most of the formulae studied provide amounts close to the RDA. The only formulae which supply intakes higher than recommended are hypoallergenic preparations, during the first year of life. In spite of this fact, Jochum et al. (15) have shown a lower selenium status in infants fed on partially

hydrolysed whey formula with no clinical sign of deficiency in comparison with human milk or formula fed infants. The difference might be caused by a reduced bioavailability of selenium from hydrolysed formula used in this study. Similarly, the follow-up formulae themselves supply enough selenium to guarantee the minimum daily intake.

As is shown in Figures 2 and 3, starter infant formulae, formulae without lactose, and formulae for children with inborn errors of metabolism, fail to provide RDA of selenium during the first month of neonate life, and those infants fed with soya formulae receive intakes that do not reach the estimated requirements during the lactating period (40 - 79 %).

Preterm intakes were calculated taking into account infant weight and paediatric specifications or manufacturers' guidelines. However it is necessary to point out that the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) does not designate the selenium amount necessary in preterm infant formula (7) nor does the NCR estimate a specific daily dietary recommendation for premature or low weight infants, in spite of the fact that infants born prematurely have decreased hepatic stores and plasma selenium concentrations compared to full term infants at birth. On the basis of limited information, it was suggested that the recommended dietary selenium intake for premature infants must be the same as that for full term infants (2.1 μg/Kg day) (37).

Figure 4 compares the daily selenium intake for infants fed on preterm formulae and breast milk, with this selenium recommendation. Both preterm formulae and human milk samples provide adequate selenium to meet the infants' requirement during the first stage of life. In most cases, intakes provided by studied preterm formulae are higher as compared with starter formulae for full term infants.

In order to establish a comparison between the daily intake provided by human milk and the different types of infant formulae considering the protein source used, a statistical analysis (Mann Whitney U-test) was performed (Table 8). Selenium intakes were calculated assuming an intake of 800 mL of breast milk or formula per day. Soy, whey, casein and skim-milk-based formulae supply a selenium intake significantly lower than human milk samples. Several infant formulae analysed provide significantly less selenium than 10 μg/day; furthermore, some do not meet the basal and normative requirements defined by Joint FAO/IAEA/WHO (6). Whole milk based, whey and casein hydrolysed formulae provide higher intrinsic selenium intake, closer to that calculated in human milk. But even so, none of the formulae analysed provide more than the suggested safe upper limit of 45 μg/day. Figure 5 shows the theoretical dietary selenium intake,

assuming an ordinary feeding regimen with the different infant formulae studied and using the doses recommended by manufactures and paediatricians.

According to these data, on an overall view and just as was expected, infants fed on the infant formulae analysed here have an intake which is between the basal and normative requirements. This might be considered to provide an adequate selenium intake. However, it is worth emphasising that several formulae included in this study could provide a deficient intake, even as litle as a third or quarter of the levels set by NCR.

Selenium interactions with other trace elements

Manufacturers have been gradually changing the protein profile of infant formula to reflect more closely human milk contents. Caseins have been replaced with high levels of whey proteins. This leads to a reduction of intrinsic selenium value from proteins (40, 41).

Because of these variations in infant formula composition and their potential impact on selenium content, the selenium level was analysed with regard to the different protein sources. Analysis of variance (one factor Anova repeat measures – Schefe F-test) showed significant differences in selenium values determined in the infant formulae investigated ($p < 0.001$). Next, Mann Whitney U-test was used to compare selenium values of different distributions of infant formulae according to protein source. The results of the this statistical analysis are presented in table 9.

The statistical test reflects the values and tendencies observed before. Firstly, selenium content in human milk is significantly higher than that found in most studied infant formula (15, 25, 29). Secondly, selenium distribution is significantly different in the studied infant formula. Thus, whole-milk-based formulae were found to contain significantly higher selenium concentrations (14.1 ± 6.3 μg/L) than skim-milk (9.5 ± 5.8 μg/L) or whey-based (8.6 ± 3.0 μg/L) formulae. The results obtained are consistent with the reported selenium distribution in cow's milk (42, 43) and with the affirmation that its concentration in formulae will depend on the relative quantity of casein used in their preparation, which is lower in the newer formulae (41).

Trace element compounds normally added to the raw material (cow's milk or soy) are supplied in an inorganic form, sometimes in a high concentration to compensate for the lower bioavailability of infant formulae (27). However, most of the manufacturers do not report the selenium content on the label. Probably, in Spanish infant formula selenium had not been added during the manufacturing process. In order to observe the possible

interactions between the selenium and different trace element contents, a comparative statistical analysis using the Spearman Coefficient was carried out (11, 12).

Statistical analysis of trace element content in infant formula yielded a significant inverse correlation between zinc and selenium in the infant formulae studied (Figure 6). The same consideration was established for premature ($p = 0.038$) and starter adapted ($p = 0.017$) formula. In cow's milk, most of the zinc is in the skimmed milk fraction and over 95 % is associated with the casein micelles (44, 45). Changes in protein profile lead to formula with low levels in selenium and high zinc concentrations.

Copper distribution in cow's milk has been reported to be 2 % in the fat fraction, 8 % bound to whey proteins, 44 % to casein and 47 % in low molecular weight ligants (46). It fits the negative interaction found in the starter adapted formulae ($p = 0.033$), since skim milk, whey and casein-based formulae show gradually lower selenium and higher copper levels, respectively (Figure 7).

However, a positive correlation was established in starter adapted formulae between selenium and aluminium concentrations ($p = 0.031$). This interaction is explained by special care taken by manufacturers in the newer formulation process (Figure 8).

Finally, a negative interaction manganese – selenium was found in whey-based infant formulae ($p = 0.026$) (Figure 9). Manganese is distributed in cow's milk as 67 % bound to casein, 18 % to low molecular weight compounds, 14 % to whey proteins and 1 % to the fat globule membrane (47). Probably this interaction corresponds to the protein separation technique used or the effect of processing conditions and handling by manufacturers.

Conclusions

ESPGAN guidelines suggest that selenium salts should not be added routinely to infant formulae. On the other hand, they recommend their inclusion in formulae designed for pre-term infants (7). In accordance with the present state of knowledge of overexposure to high selenium intake with the lack of a specific biochemical marker or where toxic selenium intake for babies is unknown (48), it is useful to pay attention to the safety range allowed between minimum and maximum selenium intake for formula fed infants.

A narrow interval of 4.5 fold between those formulae that provide inadequate or excessive selenium intake (5) and the risk of subclinical deficiency (49), especially in low birth weight or premature infants, could make it useful to standardise the selenium content of infant formula. The EEC Directive (96/4/EC) relative to infant formulae (starter and follow up formulae) lays down a maximum limit of 22.5 μg/L in selenium fortified infant formulae, and the only chemical forms allowed are selenite and selenate sodium salts (9).

In spite of everything many researchers are in favour of infant formula supplementation to ensure adequate selenium supply at levels similar to the reference standard, human milk (37). In the light of recent studies (38, 50), it is appropriate to bear in mind that all selenium sources are not equivalent, as they vary according to their nutritional bioavailability.

The need to provide infant formulae which satisfy the dietary requirement for selenium just as human milk is doing, should prompt further speciation studies (51, 52) to determine the binding form of selenium in human milk to clarify the selenium concentration and chemical form adequate to ensure optimal development of infants fed on infant formula.

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Table 1. Optimised microwave digestion program for the mineralisation of human milk and infant formulae. Analytical parameters for selenium determination.

Stage	***1***	***2***	***3***	***4***	***5***	***Ventilation***
Power *(W)*	250	0	250	400*	600*	0
Time *(min.)*	1	1	2	2	2	1

* Pulsed from power 1000 W.

Table 2. Instrumental parameters for selenium determination.

ICP-AES Parameters	
Rf forward power (W)	1000
RF generator frequency (Mhz)	42
Outer gas flow rate (L/min)	12
Nebulizer gas flow rate (L/min)	0.6
Wavelength (nm)	196.026
Integration time (s)	3.0
Internal standard voltage (V)	883
Measurement Mode	Sequential
Increment between measurements (nm)	0.0030

Table 3. Selenium content in different types of infant formulae and Spanish breast milk (μg/L)

Infant formula	*Selenium*		
	n	***Mean ± SD***	***Range***
Starter Formula			
- Non adapted	4	9.3 ± 6.0	2.9 – 17.3
- Adapted	16	9.2 ± 3.3	3.4 – 15.2
Follow-up Formula	19	11.6 ± 7.2	4.0 – 30.5
Specialised Formula			
- Without lactose Formula	7	9.9 ± 5.5	3.4 –18.7
- Hypoallergenic Formula	12	14.4 ± 9.9	5.7 – 37.2
- Inborn errors diet	10	12.9 ± 5.3	5.1 – 21.3
Soya Formula	7	6.7 ± 2.6	1.5 – 9.8
Pre-term Formula	7	15.6 ± 9.5	10.2 – 36.2
Spanish human milk	31	16.3 ± 4.7	9.4 – 29.0

Table 4. Selenium content of mature human milk from different countries

Country	*Reference*	*Se (µg/L)*	*n*	*Description*
Belgium	Robberecht et al., 1985 (13)	9.9 ± 3.4	9	2 months
Finland	Kumpulainen et al., 1984 (14)	5.8 ± 1.2	13	3-6 months (1976)
		10.0 ± 1.9	15	3-6 months (1980)
Germany	Jochum et al., 1995 (15)	9.9 ± 0.5	30	4 months
Greece	Bratakos and Ioannou, 1991 (16)	15 ± 2	16	-
Spain	Torres et al. 1999 (17)	5.2 ± 1.9	6	2 months
	Rodríguez et al., 1998 (18)	16.0 ± 1.5	7	3 months
	This work	16.3 ± 4.7	31	2-3 months
United Kingdom	Foster et al., 1996 (19)	20.6 ± 3.0	87	-
Canada	L'abbe et al., 1996 (20)	17.7 ± 3.3		2-3 months
USA	Levander et al., 1987 (21)	15 ± 1	10	3-6 months
	Litov et al., 1989 (10)	23 ± 4	12	2 months
	Smith et al., 1982 (22)	16.3 ± 4.9	20	3 months
Korea	Tamari et al., 1998 (23)	12 ± 2*	63	2 months
Japan	Tamari et al., 1998 (23)	10 ± 4*	36	2 months

*µg/kg

Table 5. Selenium levels found from different types of infant formulae

Country	*Reference*	*Se (µg/L)*	*n*	*Description*
Belgium	Rockens et al., 1985 (24)	0.6 – 4.9	13	Milk-based
Finland	Kumpulainen et al., 1987 (25)	3 – 5	42	Milk-based
France	Bougle et al., 1990 (26)	7 - 12	6	Preterm formula
Germany	Brätter, 1996 (27)	10 – 49	60	Milk-based
		16 – 45	35	Preterm formula
	Jochum et al., 1995 (15)	6.1 ± 1.9	60	Milk-based
		5.9 ± 0.6	23	HA
	Zabel et al., 1978 (28)	1.36 – 13.7*	-	Milk-based
	Lombeck et al., 1978 (29)	2.5 – 23.9*	62	Milk-based
Hungary	Gergely et al., 1991 (30)	4 - 20	-	Milk-based
Spain	Torres et al. 1999 (17)	3.8 ± 1.2	5	Milk-based
		6.1 ± 1.3	5	Follow-on
		4.5 ± 2.3	8	Soy-based
	Rodríguez et al., 1998 (18)	7.47 ± 3.69	5	Milk-based
	Alegría et al., 1995 (31)	5.3 – 15.2	8	Milk-based
		5.7 – 13.3	20	Follow-on
		15.3	2	Without lactose
		17.2	2	Soy-based
United Kingdom	Sumar et al., 1997 (32)	4.76 – 13.02	2	Adapted formula
		4.76 – 10.22	1	Casein-based
		5.18 – 13.02	1	Whey-based
		3.22 – 13.02	3	Preterm formula
		3.22 – 12.46	4	Soy-based

Saudi Arabia	Al-Saleh and Al-Doush, 1997 (33)	26 - 68	24	Milk-based
Canada	L'abbe et al., 1996 (20)	2.7 - 21	-	Unsupplem. Form.
		2.7 – 5.5	-	Soy-based
		12 – 15	-	Casein-based
		9 – 21	-	Whey-based
		4 – 15	-	Skim-milk-based
		4	-	Whole-milk-based
		16 – 35	-	Supplem. Form.
		19 – 35	-	Skim-milk-based
		16 – 23	-	Whole-milk-based
		26 - 28	-	Casein hydrolysate
USA	Smith et al., 1991 (34)	6.7 ± 1.4	20	Preterm formula
	Picciano, 1985 (35)	5 - 10	-	Milk-based
Korea	Tamari et al., 1998 (23)	9.1 ± 1.8*	18	Milk-based
		5.1*	1	Supplem. Form.
		3.7 ± 2.3*	3	Soy-based
Japan	Tamari et al., 1998 (23)	7.7 ± 3.2*	20	Milk-based
		3.8 ± 1.7*	6	HA
		4.4 ± 1.2*	4	Without lactose
		6.1*	1	Preterm formula
		5.2 ± 1.4*	3	Soy-based

*Recalculated selenium concentration and expressed in µg/L

Table 6. Selenium concentrations in different types of infant formulae with regard to the main protein content (µg/L)

Infant formula	*Selenium*		
	n	***Mean ± SD***	***Range***
Starter Formula			
- Non adapted			
Skim-milk-based	3	9.4 ± 7.3	2.9 – 17.3
Whole-milk-based	1	9.9	-
- Adapted			
Whey-based	6	7.4 ± 2.8	3.4 – 9.8
Casein-based	3	8.7 ± 1.8	7.6 –10.8
Skim-milk-based	2	6.0 ± 1.4	5.0 – 7.0
Whole-milk-based	5	12.8 ± 2.4	9.1 – 15.2
Follow up Formula			
Whey-based	1	6.1	-
Skim-milk-based	13	10.5 ± 6.5	4.0 – 26.2
Whole-milk-based	5	15.9 ± 10.3	6.1 – 30.5
Specialised Formula			
- Without lactose Formula			
Casein-based	6	10.0 ± 6.0	3.4 – 18.7
Skim-milk-based	1	9.0	-
- Hypoallergenic Formula			
Whey hydrolysed	7	17.0 ± 11.9	5.7 – 37.2
Casein hydrolysed	5	10.8 ± 5.5	6.0 – 17.4
- Inborn errors diet			
Casein-based	1	16.8	-
Skim-milk-based	1	5.1	-
Whey hydrolysed	1	13.7	-
Casein hydrolysed	4	11.9 ± 3.6	9.1 – 15.6
Free aminoacids	2	14.6 ± 6.6	9.9 – 19.2
No protein	1	7.3	-
Soya Formula			
Soy based	7	6.7 ± 2.6	1.5 – 9.8
Pre-term Formula			
Whey-based	4	11.4 ± 1.4	10.6 – 13.1
Skim-milk-based	1	10.2	-
Whole-milk-based	1	17.9	-
Casein hydrolysed	1	36.2	-

Table 7. Selenium levels (µg/L) from different types of infant formulae studied and values listed on product label.

Formula	*Powder*				*Ready to use*			
	n	***Found***	***n***	***Label***	***n***	***Found***	***n***	***Label***
Starter Formula								
Non adapted	3	10.0 ± 7.2	-	-	1	8.1	-	-
Adapted	12	9.3 ± 3.4	3	13.1 ± 4.0	4	8.9 ± 3.5	-	-
Follow up Formula	13	10.9 ± 5.6	-	-	6	13.0 ± 10.4	-	-
Specialised Formula								
Without lactose F.	7	9.9 ± 5.5	1	15.4	-	-	-	-
Hypoallergenic F.	10	14.1 ± 10.9	5	14.7 ± 0.7	2	16.1 ± 1.9	-	-
Inborn errors diet	10	12.9 ± 5.3	8	16.0 ± 10.3	-	-	-	-
Soya Formula	7	6.7 ± 2.6	2	9.0 ± 7.1	-	-	-	-
Pre-term Formula	6	16.5 ± 10.1	1	74.2	1	10.6	-	-
Total	68	11.3 ± 6.8	20	17.4 ± 15.1	14	11.7 ± 7.2	-	-

Table 8. Daily intakes of selenium with infant formulae and human milk samples assuming an intake of 800 mL per day

Human or infant milk	*Concentration (μg/L)*		*Intake (μg/day)*		*p**
Soy-based	6.7 ± 2.6	(1.5 – 9.8)	5.4 ± 2.0	(1.2 – 7.8)	< 0,001
Whey-based	8.6 ± 3.0	(3.4 – 13.1)	6.9 ± 2.4	(2.7 – 10.5)	< 0,001
Casein-based	10.3 ± 5.1	(3.4 – 18.7)	8.3 ± 4.1	(2.7 – 15.0)	< 0,01
Skim-milk-based	9.5 ± 5.8	(2.9 – 26.2)	7.6 ± 4.7	(2.3 – 21.0)	< 0,001
Whole-milk-based	14.1 ± 6.3	(6.1 – 30.5)	11.3 ± 5.0	(4.9 – 24.4)	-
Whey hydrolyzed	16.6 ± 11.0	(5.7 – 37.2)	13.3 ± 8.9	(4.6 – 29.8)	-
Casein hydrolyzed	11.1 ± 4.3	(6.0 – 17.4)	8.9 ± 3.4	(4.8 – 13.9)	-
Breast milk	16.3 ± 4.7	(9.4 – 29.0)	13.0 ± 3.7	(7.2 – 23.2)	

* Statistical comparison between human milk and infant formulae intakes (Mann Whitney U-test)

Table 9. Anova analysis of selenium distributions in infant formulae (protein source) and human milk samples

Formula	*Whey b.*	*Casein b.*	*S. milk b.*	*W. milk b.*	*W. hydrol.*	*C. hydrol.*	*Soy based*	*Free aa.*	*No protei.*	*Hum. milk*
Whey b.	X									
Casein b.	-	X								
S. milk b.	-	-	X							
W. milk b.	p < 0.01**	-	p = 0.019*	X						
W. hydrol.	p = 0.083	-	p = 0.037*	-	X					
C. hydrol.	-	-	-	-	-	X				
Soy based	-	-	-	p < 0.01**	p = 0.021*	p = 0.064	X			
Free aa.	-	-	-	-	-	-	p = 0.040*	X		
No protei.	-	-	-	-	-	-	-	-	X	
Hum. milk	p < 0.001***	p < 0.01**	p < 0.001***	-	-	p < 0.01**	p < 0.001***	p = 0.071	-	X

Se (μg/L)

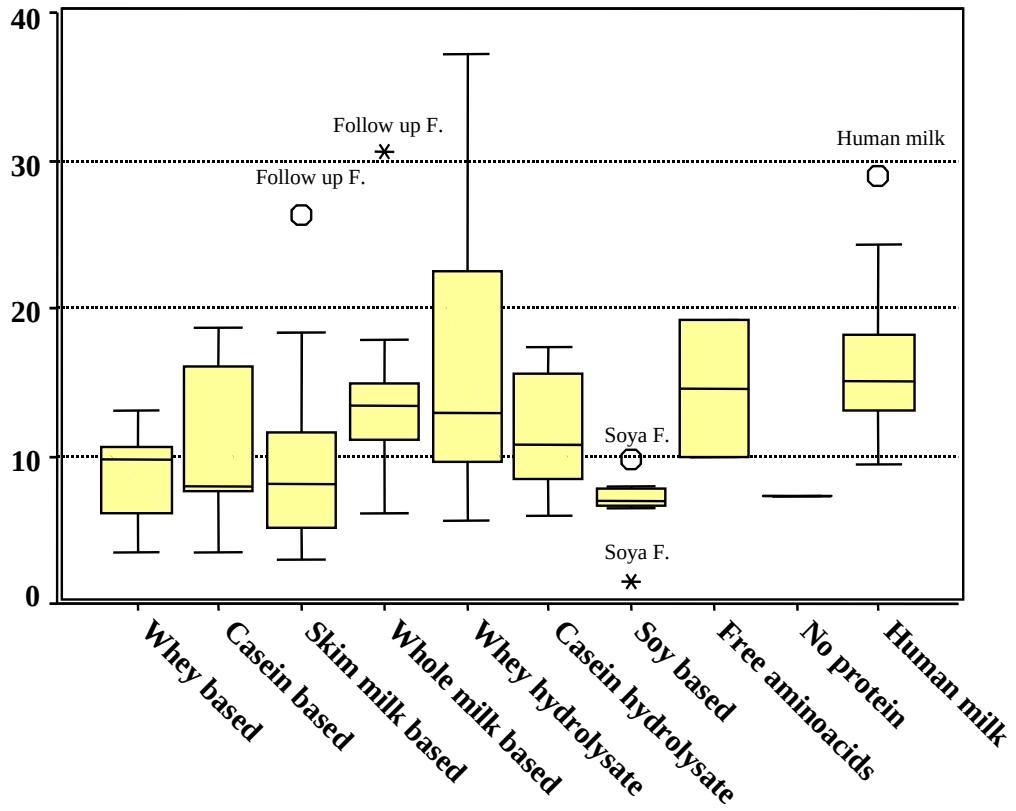
40
30
20
10
0
Follow up F.
Follow up F.
Human milk
Soya F.
Soya F.
Whey based
Casein based
Skim milk based
Whole milk based
Whey hydrolysate
Casein hydrolysate
Soy based
Free aminoacids
No protein
Human milk

Figure 1. Selenium distributions in different protein content infant formulae and Spanish breast milk (µg/L)

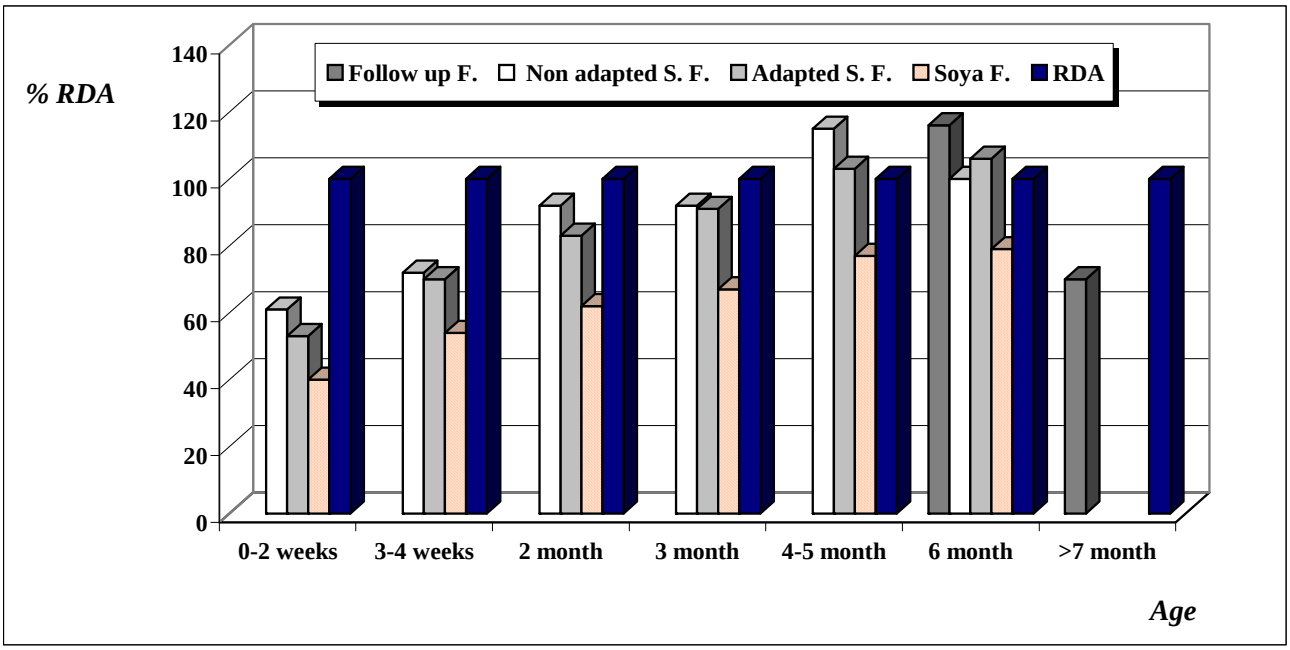
% RDA
140
120
100
80
60
40
20
0
Follow up F.
Non adapted S. F.
Adapted S. F.
Soya F.
RDA
0-2 weeks
3-4 weeks
2 month
3 month
4-5 month
6 month
>7 month
Age

Figure 2. Satisfaction percentages of RDA for selenium obtained from follow up, starter and soya formulae.

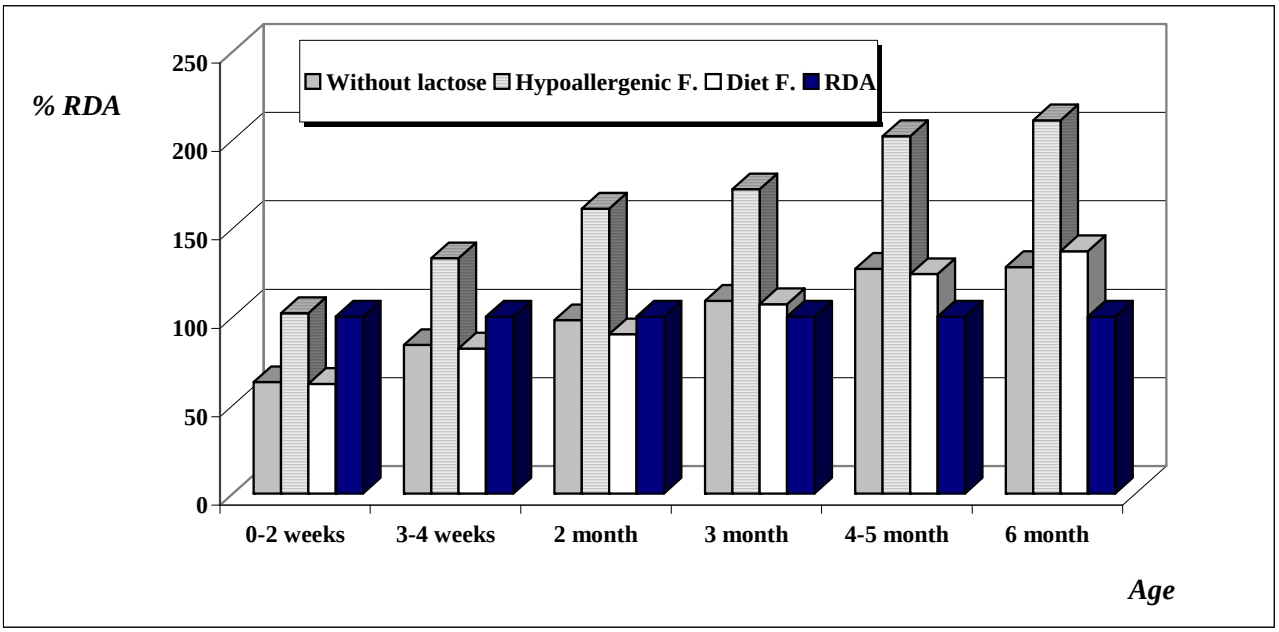
% RDA
250
200
150
100
50
0
Without lactose
Hypoallergenic F.
Diet F.
RDA
0-2 weeks
3-4 weeks
2 month
3 month
4-5 month
6 month
Age

Figure 3. Satisfaction percentages of RDA for selenium obtained from specialised formulae.

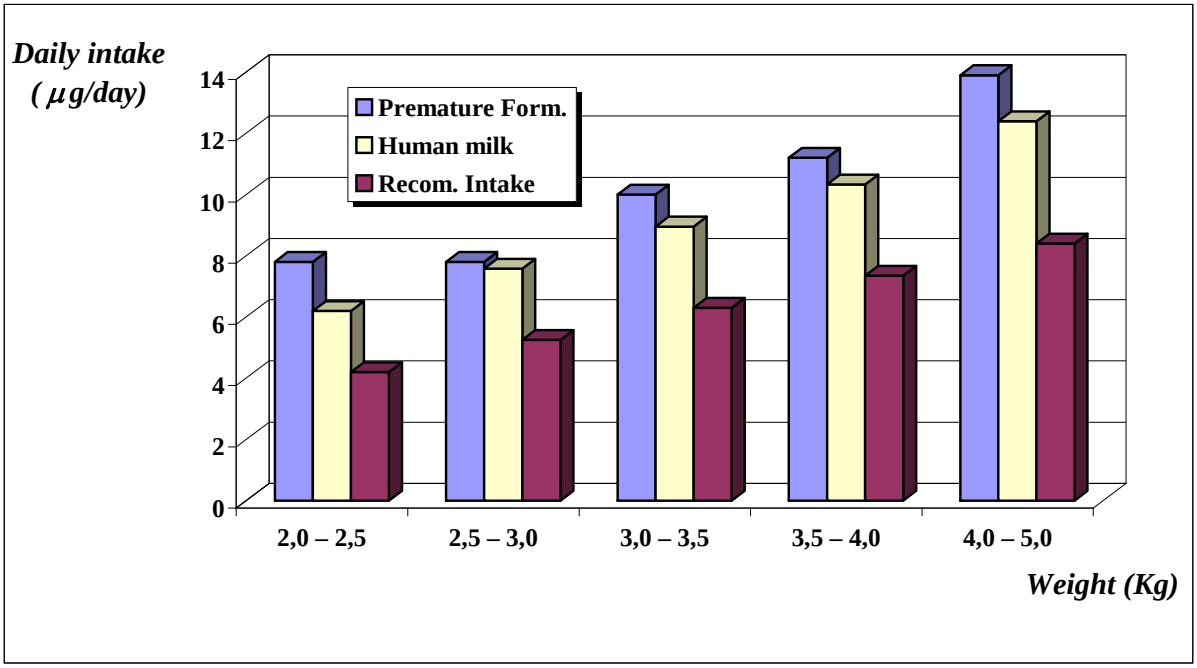
Daily intake
(μg/day)
14
12
10
8
6
4
2
0
Premature Form.
Human milk
Recom. Intake
2,0 – 2,5
2,5 – 3,0
3,0 – 3,5
3,5 – 4,0
4,0 – 5,0
Weight (Kg)

Figure 4. Daily dietary intake for infant fed on pre-term formulae and breast milk.

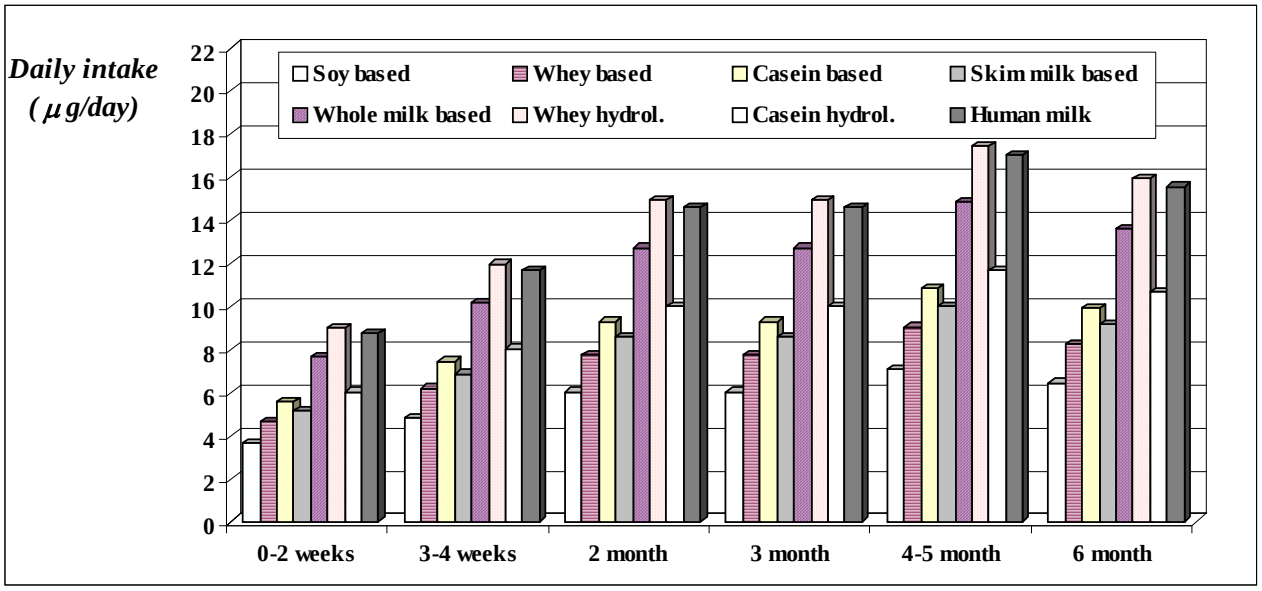
Daily intake
(μg/day)
Soy based
Whey based
Casein based
Skim milk based
Whole milk based
Whey hydrol.
Casein hydrol.
Human milk
22
20
18
16
14
12
10
8
6
4
2
0
0-2 weeks
3-4 weeks
2 month
3 month
4-5 month
6 month

Figure 5. Daily dietary intake for infant fed on infant formulae (in accordance with protein content) and breast milk.

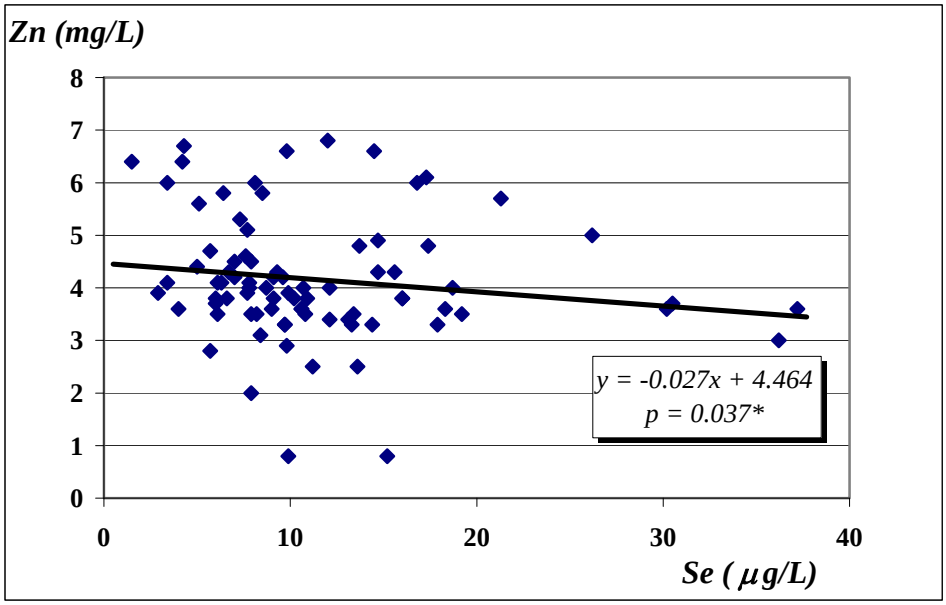
Zn (mg/L)
8
7
6
5
4
3
2
1
0
0
10
20
30
40
Se (µg/L)
y = -0.027x + 4.464
p = 0.037*

Figure 6. Zinc versus selenium in studied infant formulae.

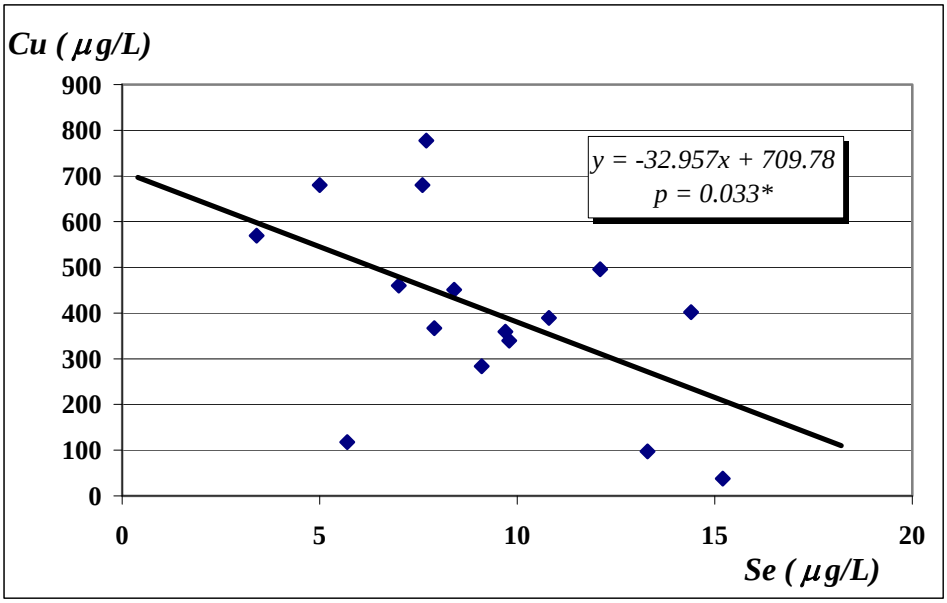
Cu (µg/L)
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
Se (µg/L)
y = -32.957x + 709.78
p = 0.033*

Figure 7. Copper versus selenium in starter adapted infant formulae.

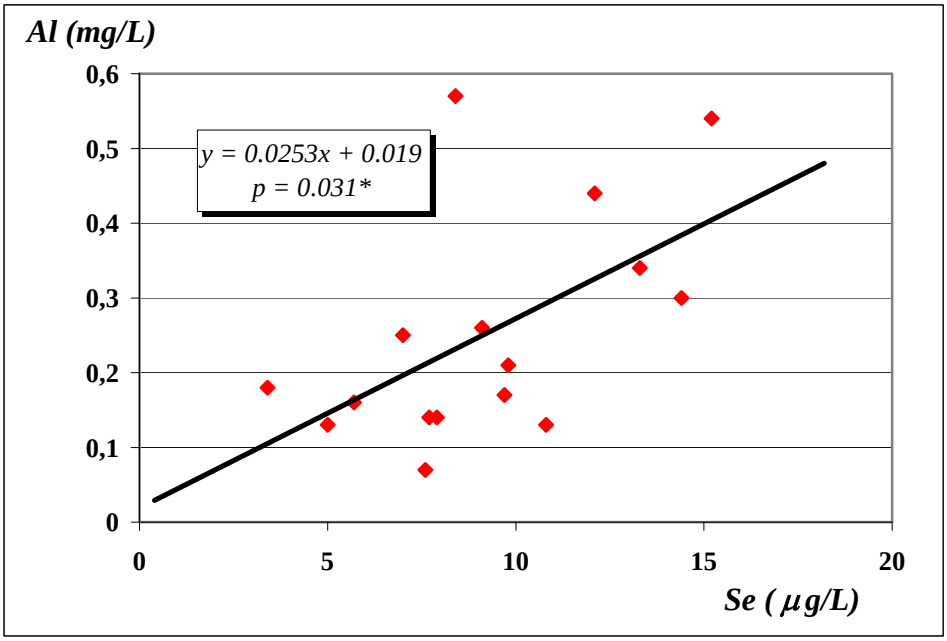
Al (mg/L)
0,6
0,5
0,4
0,3
0,2
0,1
0
y = 0.0253x + 0.019
p = 0.031*
0
5
10
15
20
Se (µg/L)

Figure 8. Aluminium versus selenium in starter adapted infant formulae.

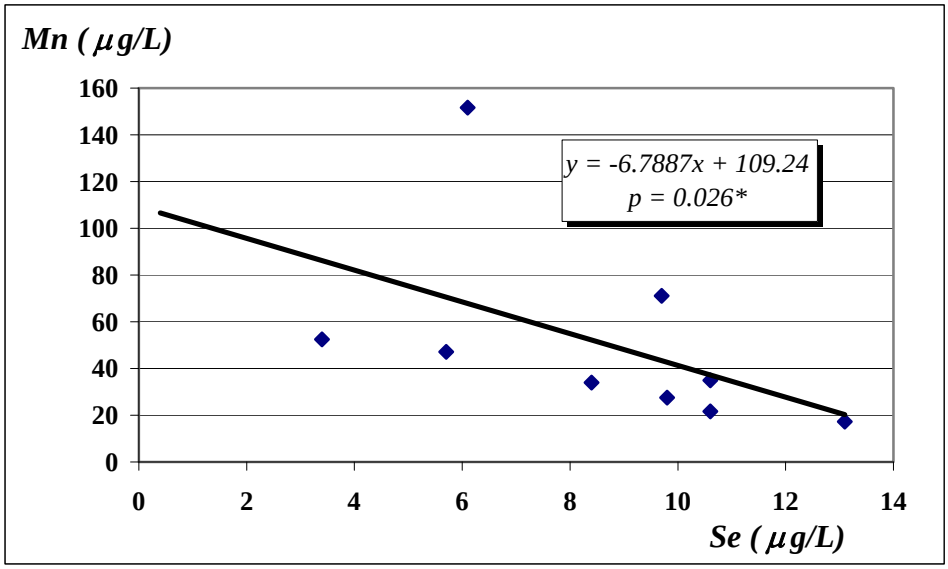
Mn (μg/L)
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
y = -6.7887x + 109.24
p = 0.026*
Se (μg/L)

Figure 9. Manganese versus selenium in whey-based infant formulae.