Review Article

Selenium in infant formula milk

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Formula-based animal milk is an alternative source of infant nutrition in many cases when breastfeeding is unacceptable or inaccessible; however, these replacements often have low selenium levels. The composition of infant formula milk should be as close as possible to that of human breast milk, both in content and chemical speciation. Selenium is an essential trace element for infants. Generally, human breast milk is the ideal food to ensure adequate infant Se intake. However, to date, sodium selenite or sodium selenate has been used as selenium supplementation in infant formula milk in most countries. This inorganic Se, which is not a natural component of food, may not be the optimal speciation for Se supplementation in infant formula milk. Advances in speciation in foods, especially in animal milk, suggest that future proposals for selenium speciation in human breast milk can lead to discussions regarding the most favorable methods of selenium supplementation in infant formula milk.

Key Words: selenium speciation, infant formula milk, human breast milk, food, policy

INTRODUCTION

Selenium (Se) is an essential trace element for both human and animals. Se is often found in the form of selenoprotein and is involved in various biochemical and physiological functions in mammalian systems, such as the enhancement of immunity and oxidation resistance.[1] Se deficiency may lead to endemic disease, typically Keshan disease or Kaschin–Beck disease. Because Se has been found to be an essential nutrient for the prevention of liver necrosis in vitamin E-deficient animals, Se has been extensively studied, particularly in the livestock industry. In the 1970s, the etiology of Keshan disease was demonstrated to be associated with severe Se deficiency in China. Subsequently, several studies on Se deficiency in infants, especially preterm infants, have reported that the condition might cause growth retardation and acute diseases in childhood as well as potentially long-term negative outcomes in adults.[2] Human breast milk and infant formula milk are two major sources of Se for infants.

Exclusive breastfeeding provides several benefits including protection against childhood infections and malocclusion, intelligence gains, and potential reduction of the risk of overweight and diabetes. Blood Se concentrations generally increase from birth until the age of 6 months in breastfed infants and then remain stable in the long term.[3] By contrast, a study reported that infants fed on formula milk without Se supplementation had decreased blood Se concentrations several months after birth.[4] The low plasma concentrations of Se and glutathione peroxidase at birth may result in early exposure to excessive oxidative stress.[5] Therefore, compared with infant formula milk, human breast milk is the ideal source of Se intake for infants.

Infant formula milk can be used as a substitute for breast milk for infants in cases of insufficient or no human breast milk intake. Infant formula milk is often based on cow's milk. However, the endogenous Se content in cow's milk is lower (only 50%–70%) than that in human breast milk.[6-8] Therefore, additional Se supplementation is required for infant formula milk.

An appropriate recipe for infant formula milk should mimic the composition of human breast milk from healthy, well-nourished women, not only in terms of the content but also the nutrients whenever possible.[9] Human breast milk is also the ideal model for the chemical form of Se chosen for supplementation in infant formula milk. The major Se species in human breast milk should be

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identified. Then, one or more of the identified species should be used in infant formula milk. However, studies on Se speciation in whole human breast milk are lacking. A potential reference for the source of Se intake may be found in the advanced analytical studies on infant supplementary food.

This review focuses on the current policies on Se supplementation in infant formula milk in different countries and regions; studies on Se speciation in food, cow's milk, and human breast milk; and analytical methods of Se speciation.

Current policies on Se Supplementation in infant formula milk

Since the 1950s, the focus of researchers in the nutrition field on Se intake increased due to Keshan disease. The disease was discovered in the winter of 1935 in northeast China. Extensive cross-sectional epidemiological studies have reported that the low Se concentration in cereal grains and the low Se status of local residents were associated with the occurrence of the disease.[10] Subsequently, in the 1980s, Se was recognized as an essential trace element for humans, especially for infants.

On the basis of this evidence, standards for Se supplementation in infant formula milk were gradually established. In a report published by the Codex Committee on Nutrition and Food for Special Dietary Uses in 1998, Se was first recommended as one of the essential nutrients in infant formula, with a maximum concentration of 7 μg/100 kcal. However, no minimum concentration was proposed by the Codex Alimentarius Commission (CAC). In 2005, an international expert group suggested that infant formula prepared ready for consumption should contain Se at the minimum and guidance upper levels of 1 and 9 μg/100 kcal, respectively. This was subsequently adopted by the CAC for its *CODEX STAN 72-1981 (Revision 2007)* and has not been changed to date. The *CAC/GL 10-1979* did not mention Se species supplementation in infant formula until it was revised in 2008. Sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4), and sodium hydrogen selenite ($NaHSeO_3$) were permitted to be added into infant formula milk.

Following the CAC, China, the European Union (EU), the United States (USA), and Australia and New Zealand (ANZ)instated their own policies on Se supplementation in infant formula milk in 1999, 2002, 2010, and 2015, respectively (Table 1). Only inorganic Se is currently permitted as supplementation in infant formula milk in most countries and regions, except for Australia and New Zealand, which allow the use of selenomethionine (SeMet).

Natural species of Se in food

Inorganic selenocompounds principally occur in the form of Se (IV) and Se (VI) in water and vegetables and in the form of hydrogen selenide (H2Se or HSe−) in animal foods. Organic selenocompounds are widely distributed in different food types including fungi, vegetables, and animals. They are mainly incorporated into proteins and can currently be divided into three categories: specific enzymatic proteins with selenocysteine (SeCys) incorporated into their active center, proteins containing nonspecifically incorporated Se, and Se-binding proteins. The major chemical forms of Se speciation in different food types are summarized in Table 2.[11-21]

Differences among Se species in ecosystems

Se speciation varies in the environment and various creatures (Figure 1).

Soil layer: Most Se exists in an inorganic form in soils and is derived from anthropogenic (fossil fuel burning, metal smelting, and ship emissions) and natural sources (biomethylation, rock weathering, and volcanic activity).[22,23] Some microorganisms in soil can transform inorganic Se into organic Se, accounting for a small proportion of Se in soil.[14]

Plant layer: In the natural condition, Se absorbed by plants is mainly transformed into organic Se speciation.[13,14] Recently, spraying inorganic Se fertilizers has become a common agricultural practice.[24] Such exogenous Se significantly and rapidly improves Se content in plants, resulting in higher proportions of inorganic Se.[25,26]

Animal layer: Plants are the food source for herbivores, whereas some small animals are the food source for carnivores. Therefore, compared with plant foods, natural animal-derived foods contain smaller quantities of inorganic Se. Sometimes, human intervention may result in animals having a higher intake of inorganic Se, such as through the use of inorganic Se as a supplement for dairy cows.[27]

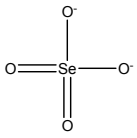
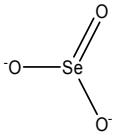
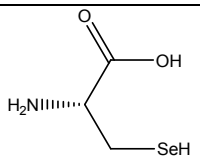
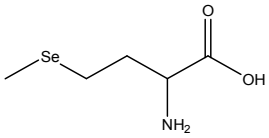
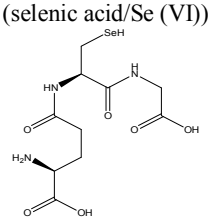
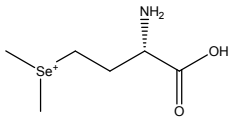
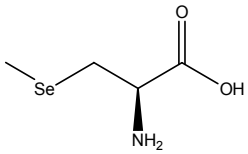
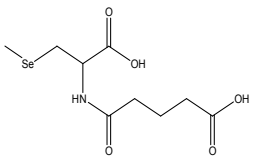
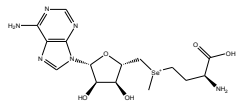
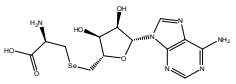
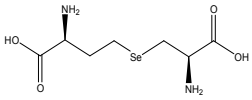
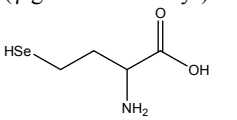
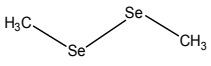
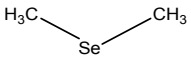
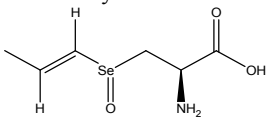
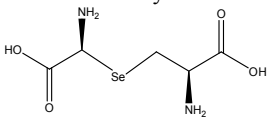
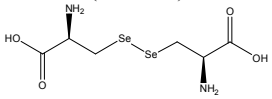
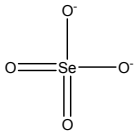
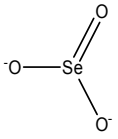
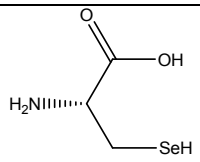
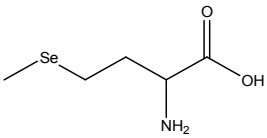
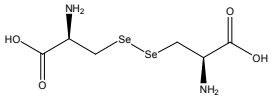
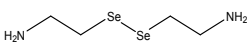
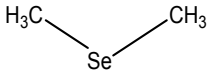
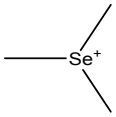
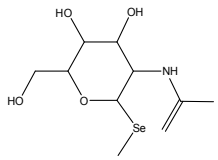
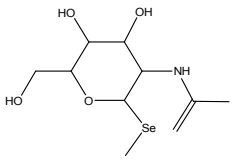
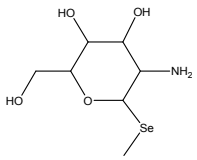
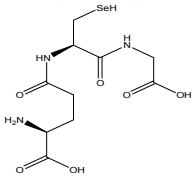
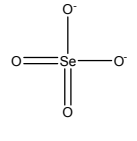
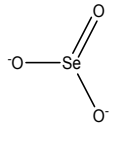
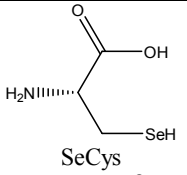
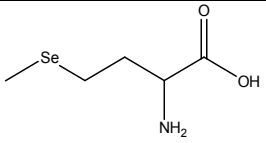
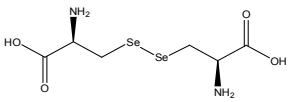
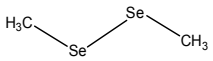
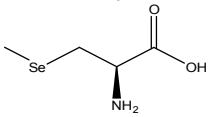
Human layer: Human diets consist of plants (crops, vegetables, and fruits); animals (domestic animals); and a

Table 1. Se level and supplementation in infant formula milk in different countries or regions

Countries or regions	Minimum and Maximum or GUL (μg/100 kcal)	Supplemental Se speciation	Standard	Year
CAC	1-9 (GUL)	Na_2SeO_3, Na_2SeO_4, $NaHSeO_3$	CODEX STAN 72-1981, CAC/GL 10-1979	1998
EU	1-9	Na_2SeO_3, Na_2SeO_4	Directive 2013/46/EU	1999
ANZ	1.05-4.98	SeMet, Na_2SeO_3, Na_2SeO_4	Standard 2.9.1	2002
China	2.01-7.95	Na_2SeO_3, Na_2SeO_4	GB 10765-2010, GB 14880-2012	2010
USA	2-7	-	CFR Part 107	2015

"-" indicates that the standard on this part is absence.
"Year" indicates that the year in which Se firstly recommended as a required nutrient in this country or region.

Table 2. The major natural species of Se in food

Se speciation in vegetable food			
 Selenite (selenic acid/Se (VI))	 Selenite (selenous acid /Se (IV))	 Selenocysteine (SeCys)	 Seleno-L-methionine (SeMet)
 GSeH	 Se-methylselenomethionine	 Methylselenocysteine (Se-MetSeCys)	 γ-glutamyl-Se-methyselenocysteine (γ-glu-Se-MetSeCys)
 Se-Adenosylselenomethionine	 Se-Adenosylselenocysteine	 Se-Cystathionine	 Se-Homocysteine
 Dimethyl diselenide (DMDSe)	 Dimethyl selenide (DMSe)	 Se-propenylSeCysteine-Se-oxide	 Se-Cystathione
 Selenocystine (SeCys2)			
Se speciation in animal food			
 Selenite	 Selenite	 SeCys	 SeMet
 SeCys2	 Selenocystamine (SeCysA)	 DMSe	 Trimethylselenonium ion (TMSe+)
 Se-Me-GalNAc	 Selenosugar 1	 Selenosugar 3	 GSeH
Se speciation in fungi food			
 Selenite	 Selenite	 SeCys	 SeMet
 SeCys2	 DMDSe	 Se-MetSeCys	

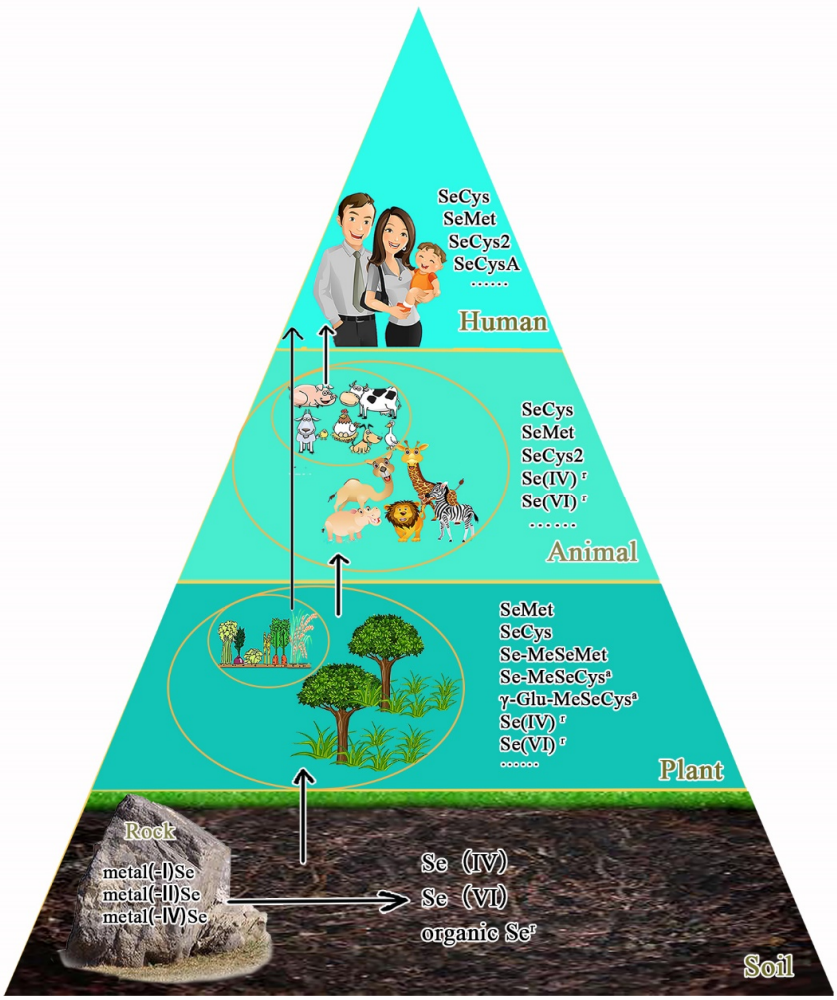


Figure 1. Se speciation varies in different layers of ecosystem. There are four layers in pyramid, respectively representing soil, plants, animals, human beings from bottom in sequence. Black arrows indicate processes that Se transfers form environment to plants, then animals or human beings, ultimately human beings. The major selenium speciation is pointed out in each layer. Superscript "a" implies that only occurs in Se-accumulator plants and "r" implies rare.

small amount of microorganisms (mushrooms and yeast). Thus, only organic Se species are naturally found in human beings.[13,28] When humans consume inorganic Se-enriched crops or animals or receive inorganic Se supplementation, inorganic Se may be introduced in the human body including in human breast milk.

Together, the proportion of organic Se gradually increases from the bottom to the top of the food chain, whereas that of inorganic Se increases from the top to the bottom of the food chain. Thus, to increase the total Se intake in human diets, inorganic Se is sometimes introduced into the food chain through human intervention; this phenomenon is opposite to the result of natural selection.

Analytical methods of Se speciation in food

Sample pretreatment

Sample pretreatment usually involves techniques such as enzymolysis[29,30] and water extraction.[31] In each investigation, appropriate methods must be explored depending on the distinctive properties of the various samples involved.

Separation technique

Liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE), and multidimensional separation techniques have often been used in the analysis of Se speciation.[32-35] LC is the most used separation technique; it has been reported in nearly 70% of relevant studies. According to different separation principles, it can also be classified as size exclusion chromatography (SEC), ion chromatography (IC), ion exchange chromatography (IEC), and reversed-phase chromatography (RPC). SEC, alone or in combination with other separation techniques, has been a convenient technique for the separation of macromolecular Se compounds, such as selenoprotein and Se polysaccharide.[36,37] SEC provides several advantages such as the association of elements with different molecular weight fractions. IEC and RPC are mainly applied for the separation of micromolecular Se compounds, such as SeMet, SeCys, Se (IV), and Se (VI).[16,38]

GC is rapid and features high resolution. Volatile Se compounds, such as DMSe and DMDSe, can be directly separated through GC,[33] whereas nonvolatile compounds must be converted into volatile compounds in advance through derivatization.[39]

The main attributes of CE are high efficiency, versatility, and low cost. This technique offers the ability to separate a large number of electrically charged and noncharged compounds within a wide range of molecular

weights in a single run and a relatively short time.[40] In addition, CE has been classically employed for separating amino acids and thus can be a favorable choice for resolving selenoaminoacid mixtures.

Analytical techniques

Inductively coupled plasma mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS), atomic absorption spectroscopy (AAS), and mass spectrometry (MS) are often used in the detection of Se speciation in various samples.

ICP-MS is the most commonly used detection technique, employed in nearly 80% of relevant reports, and has been applied for Se speciation analysis in food because of its sensitivity, robustness, and easy hyphenation with HPLC.[41] However, ICP-MS had one crucial disadvantage: because it provides only elemental information about Se-containing metabolites, it is effective only when the retention time of samples matches that of certified or authentic Se species (standard compounds). Additionally, if different Se species have the same retention time, they cannot be separated unless isotope labeling is applied.

AFS is a highly developed method. Recently, the Chinese Academy of Agricultural Sciences reported that four Se species, Se (IV), Se (VI), SeMet, and Se-methylselenocysteine (Se-MetSeCys), were simultaneously determined in Se-enriched yeast through LC-hydride generation-AFS.[42]

AAS features high accuracy, high selectivity, and rapid analytical capability. Tuzen et al developed a rapid and environmental friendly method for the speciation of inorganic Se in beverages and total Se in food samples by using graphite furnace AAS.[43]

Electrospray ionization or atmospheric pressure chemical ionization mass spectrometry (ESI/APCI-MS) is a milder technique than ICP-MS. Current ESI/APCI-MS involves several types of mass filters and sectors, such as quadrupole mass spectrometry (QMS), time-of-flight mass spectrometry (TOF-MS), and ion trap (MSn) as well as combinations such as tandem QMS and a hybrid of QMS and TOF-MS (Q-TOF-MS), which enable more precise structure elucidation than single mass spectrometers do. Recently, ESI/APCI-MS has been used to detect organic Se in food and was reported to have a lower detection limit of 0.5 ng/mL.[44]

Se speciation in human breast milk

Se species in the milk of mammals

According to previous studies, Se speciation in the milk of different mammals is as follows: selenocystine (SeCys2), SeCys, SeMet, Se (IV), DMSe, and DMDSe (Table 3). Species and content have varied in these studies because of differences in animal feeding, chemical forms of Se supplementation, and analytical methods used.[32,45-51]

Advances in the analysis of Se speciation in human breast milk

In 1997, Michalke et al observed four free Se chemical forms in human breast milk, GSeH (4%–32% of the total Se amount) > selenocystamine (SeCysA) > SeCys2 > SeMet, through capillary zone electrophoresis (CZE) coupled with electrothermal vaporization ICP-MS.52 They established two methods of separating inorganic and organic Se from human breast milk by combining CZE or capillary isoelectric focusing with ICP-MS.[53] Inorganic Se was not detected in human breast milk and accounted for approximately 30% of total Se in cow milk.[45] Al-Awadi and Srikumar reported that SeMet was the major Se form associated with breast milk whey and that the SeCys content was negligible.[47]

To screen for potential major Se species in human breast milk, additional studies must focus on possible Se compounds including selenoprotein and proteins containing nonspecifically incorporated Se and Se-binding proteins.

Proposals for the analysis of natural Se species in whole human milk

The analytical methods used for Se speciation in food, especially in animal milk, can provide a solid basis for the analysis of natural Se species in whole human milk.

Appropriate sampling procedures

Human breast milk samples should be obtained from healthy lactating mothers in areas with normal Se concentrations in the soil. Human breast milk samples from lactating women in low- and high-Se-soil areas as well as those from lactating mothers provided with Se supplementation are also needed. Appropriate samples should be compared to evaluate whether any differences exist in the Se content and the proportions of major Se species.

Sample pretreatment

Milk and dairy products are O/W emulsions requiring demulsification. The enzymes chosen in the Se speciation analysis of cow milk can be used as a reference for human breast milk because of the similarities between the

Table 3. Selenium speciation in whole mammal animal milk

Mammal animal milk	Method	Se speciation	Year
Goat milk	Protein electrophoresis	GSH-Px	1984
Cow milk	Protein electrophoresis	GSH-Px	1984
Cow milk	Spectrophotometer	GSH-Px	2001
Cow milk	Enzymatic digestion- LC-UV-HG-AFS	SeCys2, SeMet	2007
Cow milk	Enzymatic digestion- HPLC–ICP-MS	SeMet, SeCys , Se(IV)	2008
Cow milk	Enzymatic digestion- HPLC–ICP-MS	SeMet, SeCys	2008
Cow milk	GC–MS-SIM	DMSe, DMDSe	2011
Mouse milk	SDS-PAGE gel	GSH-Px, Sepp	2014
Cow milk	UPLC-MS/MS	SeMet	2016

two substances. Pronase E, protease XIV, and lipase were usually employed for enzymatic digestion in the Se speciation analysis in milk.[45,48]

Separation technique

In the 1990s, CE was widely used for the separation of Se species, including those in human breast milk.[52] Currently, LC is the most frequently used separation technique in the analysis of Se speciation in milk. An LC column with a chiral stationary phase in combination with both AFS and ICP-MS was used in a previous study for the analysis of chiral speciation of SeMet in human breast milk and infant formula milk. Only the L-enantiomer of SeMet was detected, and up to 30% of SeMet is present as D-SeMet in infant formula milk.[54] Recently, GC was also used to successfully separate DMSe and DMDSe in cow milk,[32] indicating that it can be a reference for the analysis of Se speciation in human breast milk.

Analytical techniques

ICP-MS is the most commonly used detection technique in the analysis of Se speciation in milk, owing to its high sensitivity.[45] Recently, a Spanish study developed an easy and quick on-line Se speciation method (LC-UV-HG-AFS) in cow milk obtained after adding different supplementation to cow feed.[48] After organic supplementation was added in the form of selenized yeast, the milk samples presented three species of Se, namely SeCys2, Se (IV) and SeMet; whereas only SeCys2 and Se (IV) were present in milk samples obtained after adding inorganic supplementation . However, this form of analysis is unable to detect SeCys, the major ingredient of selenoprotein and GPx. ESI/APCI-MS can provide molecular information. When used in the detection of organic Se in cow milk, ESI/APCI-MS was reported to have a lower detection limit of 0.5 ng/mL.[51] Considering its application and low limit of quantification, this technique might be suitable for the analysis of organic Se speciation in human breast milk.

Risk management

Narrow safety range

Se has a narrower safety range than most other minerals. In addition to acute toxicity, cancer chemoprevention research involving Se supplementation has reported an increased risk of type 2 diabetes in individuals with high baseline Se levels.[55] Similar results were also observed in studies using animal models.[56]

Toxicity

The biological activity of Se is determined not only by its content but also its chemical species. Reports on the acute toxicity and subchronic toxicity of different Se species in different animal species during the last 30 years are reviewed and summarized in Table 4.[57-73] The toxicity of Se is correlated with its chemical form. The lethal dose and no observed adverse effect level of inorganic Se are lower than those of organic Se in the same animal species. In addition, studies have reported that clinical signs of Se toxicity, including alopecia, staggering gait, coronary band separation, and neurological signs of paralysis, are more severe and occur earlier in pigs fed with sodium selenate than in those fed with DL-SeMet.[74,75] Furthermore, cytotoxicity testing indicated that the cytotoxicity

Table 4. Acute toxicity and subchronic toxicity indices of Se

Se species	Animal species	LD_{50} (mg Se/kg)	NOAEL (mg Se/kg)	Category	Year	Ref.
Inorganic Se						
Selenite	Sheep	0.7	-	-	1987	57
Selenite	Rat	5	-	1	1988	58
Selenite	Rat	-	0.14 (male) 0.2 (female)	-	2005	59
Selenite	Mouse	15.86	-	2	2009	60
Selenite	Mouse	3.86	-	1	2010	61
Selenite	Mouse	3.86	-	1	2010	62
Selenite	Mouse	4.6	-	1	2012	63
Selenite	Mouse	-	0.048	-	2010	64
Selenite	Potworm	7.3	6.21	-	2007	65
Selenate	Potworm	0.97	4.41	-		
SeO_2	Mouse	5.23	-	2	2013	66
Organic						
SY	Dog	-	0.06	-	2006	67
SY	Rat	37.3	-	2	1988	58
SY	Rat	-	0.23	-	2006	67
SY	Mouse	26.38	-	2	2006	68
Se enriched probiotics	Mouse	18.49	-	2	2007	69
Se enriched product	Mouse	63.6	-	3	2013	70
SeMet	Mouse	25.6	-	2	2007	71
MSC	Mouse	14.6	-	2		
MSC	Mouse	12.6 (female) 9.26 (male)	-	2	2014	72
Ebselen	Mouse	687.15	-	4	2005	73

Toxicity was categorized according to the GHS classification.
"SY" indicates Se enriched yeast.
"-" indicates that the value is absent.

of inorganic Se is considerably higher than that of organic Se.[76] All these findings indicate that the toxicity of inorganic Se is higher than that of organic Se, regardless of acute toxicity, subchronic toxicity, or cytotoxicity.

Conclusion

The levels of Se supplementation in infant formula milk should be as close as possible to the levels of Se compounds in human breast milk. To date, Se compounds used in infant formula milk in most countries are different from those in human breast milk. The available evidence indicates that SeMet, SeCysA, and SeCys2 are favorable candidates for Se supplementation in infant formula milk. Additional studies should consider major Se species and specific proportions in whole human breast milk. The observed effects of nutrients on changing diet patterns[77,78] as well as the enhanced biological activity of serine or protein with selenocompounds[79,80] suggest that the relationship between Se and diet patterns among infants who consume supplementary food should be investigated in future studies.

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