

# Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the risk assessment of inadequate intake of antioxidant minerals (metals) in food supplements that can lead to prooxidant effects: copper, manganese, selenium and zinc

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## Abstract

The term antioxidant generally refers to the property of some substances to prevent oxidative damage. Oxidative stress is one of the etiopathogenic mechanisms of a large number of pathologies with high prevalence in our society, and numerous studies have been directed in recent years to demonstrate the beneficial properties of the intake of compounds with antioxidant potential, promoting and encouraging their consumption. Therefore, consumer interest in the use of substances with antioxidant properties and the supply of foods and food supplements with alleged antioxidant

effects have increased considerably. On the other hand, some antioxidants have shown contradictory behaviours since, in certain doses or conditions, they can become prooxidants.

The divalent metals copper, manganese, selenium and zinc have been demonstrated to contribute to the protection of cells against oxidative damage by acting as cofactors of antioxidant enzyme systems. These minerals have approved health claims related to the protection of cells against oxidative damage under the European regulatory framework.

The supplementation of copper, manganese, selenium and zinc through fortified foods and food supplements in the healthy adult population is safe under the conditions laid down by European regulations. On the other hand, scientific literature suggests that these metals could have prooxidant effects in certain doses or conditions. It is recommended that studies be carried out with an adequate methodology to evaluate these effects thoroughly and under which conditions they occur, as well as the adverse effects that could derive from them.

In addition, for pregnant or lactating women, children, the elderly and people with certain pathologies, supplements should only be taken after consultation with a health professional since there is little information on the safety of many substances in these stages and, sometimes, there may be interactions with medications. In no case should they replace the use of medicines without proper medical supervision.

The maximum amounts of minerals that could be used in food supplements per daily intake as recommended by the manufacturer, as well as the amount that could be added to foods, should be established taking into account the maximum safe levels of vitamins and minerals and the intake of vitamins and minerals from other food sources, as already provided for in Directive 2002/46/EC on food supplements and Regulation (EC) No. 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods.

## Key words

Metals, minerals, copper, manganese, selenium, zinc, antioxidant, prooxidant, food supplements.

## Suggested citation

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## 1. Introduction

The term antioxidant generally refers to the property of some substances to prevent oxidative stress. Although there is evidence that a diet rich in fruits and vegetables is healthy and prevents various diseases, it is not clear whether this is related to the number of antioxidants in these foods or to other factors. On the other hand, food supplements containing antioxidants have not demonstrated a decreased risk of developing cardiovascular disease or cancer (NCCIH, 2013) (Aune et al., 2018).

However, in recent years, both consumers' interest towards the intake of substances with antioxidant properties and supply of foods and food supplements with purported antioxidant effects from the food industry have significantly increased. As an example of the above, of 509 food supplements notified in Spain from 2007 to 2021 containing "antiox" or "detox" in their trade name, around 40 % were notified in the last 5 years. Many of these supplements contain divalent metals (copper, manganese, selenium and zinc) in their composition, as well as other compounds and extracts from various plants.

Numerous studies have been conducted in recent years to demonstrate the beneficial properties of the intake of potentially antioxidant compounds, promoting and encouraging their consumption. In this regard, a large number of health claims of different substances, based on their antioxidant or protection capacity against oxidative stress, have been submitted in the European Union under Regulation (EC) No. 1924/2006 (EU, 2006a) on nutrition and health claims made on foods. However, only the declarations of 8 of these substances have a favourable report from the European Food Safety Authority (EFSA).

On the other hand, some antioxidants have shown contradictory behaviours since they may become prooxidants in certain doses or conditions. In this regard, in previous reports on the usage conditions for certain substances in food supplements, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has warned that, it should be noted that under certain conditions, such as the intake of high doses, changes in pH or the presence of certain substances, some substances with antioxidant action might behave as prooxidants (AESAN, 2012, 2013, 2014, 2015, 2017).

In addition to the fact that food supplements containing antioxidants, although specifically regulated, are freely available, that a large part of the population is very prone to their consumption, and that there is also a large market for foods enriched with this type of substances, the daily diet-food supplements-enriched foods contribution could make it easier to exceed the safety limits, resulting in a change from a benefit to a harm of the consumer's health. In short, the doses provided by food supplements are generally much higher than those provided by fortified foods.

In a non-exhaustive sampling carried out on food supplements containing the words "antiox" or "detox" in their commercial name, registered in the General Health Registry for Food Companies and Foods (RGSEAA), the composition of the food supplements registered in 2019, 2020 and 2021 (98 in total) has been analysed. Among these products, some contain a mixture of plant extracts, and others contain vitamins and minerals (alone or together with plant extracts). Among the latter, some contain divalent metals (32 % of the 98).

Therefore, AESAN has requested the Scientific Committee to assess the risk of inadequate intake of antioxidant minerals as food supplements that can lead to prooxidant effects: copper, manganese, selenium and zinc, to determine whether recommendations can be made in this regard, given the characteristics of the diet of the population in Spain.

## 2. Health claims of substances with a purported antioxidant effect

As indicated above, a demonstration of the interest in substances with a purported antioxidant effect are the numerous health claims submitted for authorisation under Regulation (EC) No. 1924/2006 (EU, 2006a).

This Regulation aims to ensure that health claims are truthful, clear, reliable and useful to the consumer when choosing a healthy diet and therefore regulates nutrition and health claims that can be used in the labelling, presentation and advertising of foods placed on the market in the European Union.

In this regard, Article 5 of said Regulation provides that the use of nutrition and health claims shall be authorised only if certain general conditions are met, e.g. the presence, absence or reduced content in a food or category of food of a nutrient or other substance in respect of which the claim is made has been shown to have a beneficial nutritional or physiological effect, as established by generally accepted scientific data. Certain conditions concerning the nutrient or substance for which the claim is made should also be met, referring to its content in the final product or whether it is in a form that is available to be used by the organism (EU, 2006a).

Health claims are only allowed if certain information is included in the labelling or, if there is no labelling, in the presentation and advertising (EU, 2006a):

- a) a statement indicating the importance of a varied and balanced diet and a healthy lifestyle;
- b) the quantity of the food and pattern of consumption required to obtain the claimed beneficial effect;
- c) where appropriate, a statement addressed to persons who should avoid the consumption of the food; and
- d) an appropriate warning for products that are likely to present a health risk if consumed excessively.

Applications for authorisation of such declarations are submitted to the competent national authorities of each Member State of the European Union, which forward them to EFSA for assessment and opinion. This opinion is sent to the European Commission and the Member States for a decision on the application. These health claims were approved under Article 13 of Regulation (EC) No. 1924/2006 (EU, 2006a), in Commission Regulation (EU) No. 432/2012, establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health (EU, 2012).

At the time of writing this report, there are 277 authorised health claims.

As regards health claims referring to the protection against oxidative stress of certain substances, EFSA concluded that claims based on the *in vitro* free radical removal capacity refer to a pro-

perty measured in models, and it has not been established to have a beneficial physiological effect on humans, as required by Regulation (EC) No. 1924/2006 (EU, 2006a). Therefore, health claims presented under Article 13 of said Regulation, after a thorough study and debate within the Working Group of the Commission, were authorised in terms of “protection against oxidative stress”, but not with the terms “antioxidant,” “pro antioxidant” or similar (EFSA, 2017). Of the 277 health claims with a favourable EFSA report and that have been authorised, 8 relate to protection against oxidative stress of certain substances. Among these claims, those referring to divalent metals are reported in Table 1. Regulation (EU) No. 432/2012 (EU, 2012) sets out several conditions for the use of each of these claims. In particular, for copper, manganese, riboflavin, selenium, vitamin C, vitamin E and zinc, it is provided that the claim referring to the contribution of the protection of cells against oxidative stress may only be used in respect of foods that are at least a source of those nutrients as referred to in the claim “Source of [name of vitamins] and/or [name of minerals]” as listed in the Annex to Regulation (EC) No. 1924/2006 (EU, 2006a), i.e. the product must contain at least a significant amount of vitamins or minerals as defined in Annex XIII to Regulation (EU) No. 1169/2011 (EU, 2011) (at least 15 % of the nutrient reference values and supplied by 100 g or 100 ml or per portion if the package contains a single portion) or an amount established by the derogations granted under Article 7 of Regulation (EC) No. 1925/2006 (EU, 2006b).

**Table 1.** Health claims concerning protection against oxidative stress authorised in the European Union for minerals/divalent metals (EU, 2012)

Substance	Authorised health claims related to protection against oxidative stress	No. of applications submitted to EFSA	EFSA opinion
Copper	Copper contributes to the protection of cells from oxidative stress	2	EFSA (2009a)
Manganese	Manganese contributes to the protection of cells from oxidative stress	1	EFSA (2009b)
Selenium	Selenium contributes to the protection of cells from oxidative stress	10	EFSA (2009c) EFSA (2010)
Zinc	Zinc contributes to the protection of cells from oxidative stress	2	EFSA (2009d)

Other claims concerning substances with purported antioxidant effects and that have not received a favourable opinion referred to anti-ageing properties or skin protection against photo-oxidative stress induced by ultraviolet radiation. The 20 applications that referred to a purported antioxidant effect have been rejected by EFSA for various reasons, including that the claim was based on the ability to inhibit (scavenging) free radicals *in vitro*, but this ability had not been shown to exert a beneficial physiological effect on humans, or no cause-and-effect relationship had been demonstrated between the consumption of these substances and the beneficial physiological effect.

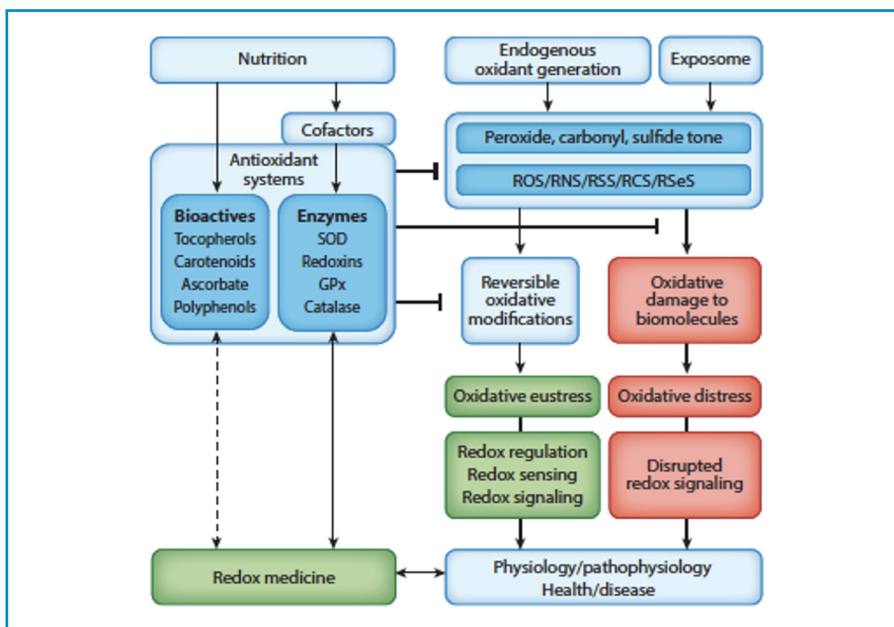
Health claims authorised for copper, manganese, selenium and zinc under Regulation (EU) No. 432/2012 (EU, 2012) are included in Table 2.

**Table 2.** Health claims authorised for copper, manganese, selenium and zinc under Regulation (EU) No. 432/2012

Claim	Copper	Manganese	Selenium	Zinc
Contributes to normal spermatogenesis			x	
Contributes to normal fertility and reproduction				x
Contributes to the normal formation of connective tissue		x		
Contributes to normal cognitive function				x
Contributes to the normal thyroid function			x	
Contributes to normal skin pigmentation	x			
Contributes to normal hair pigmentation	x			
Contributes to the protection of cells from oxidative stress	x	x	x	x
Contributes to normal DNA synthesis				x
Contributes to normal protein synthesis				x
Contributes to normal acid-base metabolism				x
Contributes to the normal function of the immune system	x		x	x
Contributes to normal functioning of the nervous system	x			
Contributes to the maintenance of normal skin				x
Contributes to the maintenance of normal vision				x
Contributes to the maintenance of normal nails			x	x
Contributes to the maintenance of normal bones		x		x
Contributes to the maintenance of normal testosterone levels in the blood				x
Contributes to the maintenance of normal hair			x	x
Contributes to maintenance of normal connective tissues	x			
Contributes to normal energy-yielding metabolism	x	x		
Contributes to normal metabolism of vitamin A				x
Contributes to normal metabolism of fatty acids				x
Contributes to normal carbohydrate metabolism				x
Contributes to normal macronutrient metabolism				x
Has a role in the process of cell division				x
Contributes to normal iron transport in the body	x			

### 3. Biochemical bases of oxidative stress

Oxidative stress is a process that occurs in our body due to an excess of free radicals and the lack of antioxidants to counteract them. That is, it is an imbalance between the production of Reactive Oxygen Species (ROS) and the ability of a biological system to rapidly neutralise intermediate reagents or repair the resulting damage (Pizzino et al., 2017). Figure 1 shows the cascade of the disturbance in the antioxidant-prooxidant balance (Sies et al., 2019).



**Figure 1.** Cascade of disturbance in the antioxidant-prooxidant balance. Cofactors include micronutrients and metal ions. Redoxins are thioredoxin, glutaredoxin and peroxiredoxin systems. The generation of endogenous oxidants includes the respiratory chain, lipid oxidations and others. Abbreviations: GPx, Glutathione Peroxidase; RCS, Reactive Carbonyl Species; RNS, Reactive Nitrogen Species; ROS, Reactive Oxygen Species; RSeS, Reactive Selenium Species; RSS, Reactive Sulphur Species; SOD, Superoxide Dismutase. **Source:** (Sies et al., 2019).

These are chemical species that contain one or more electrons, that perform their orbital track joint on an uneven number basis, provoking a great instability in the molecular equilibrium, only restored when they manage to subtract from their vicinity the electron required to constitute the essential electronic parity. Among the main Reactive Oxygen Species are superoxide anion ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical ( $OH^{\cdot}$ ), singlet oxygen ( $^1O_2$ ) and hypochlorous acid (HClO).

A compound is transformed into a free radical when it loses an electron, when it gains an electron, and in the symmetrical fission of covalent compounds, where each fragment retains an odd electron, as typically occurs in lipoperoxidation. This situation generates an energetic instability, a circumstance that makes them tend to react with other molecules by capturing electrons (oxidising) and generating a new instability in them, which can trigger a chain reaction (Cheeseman and Slater, 1993).

Under normal conditions in aerobic organisms, the loss of activated oxygen from the mitochondria during the normal functioning of oxidative breathing is the most crucial source of this type of substances. This phenomenon occurs at the electron transport chain level, the last stage of high-energy protons' production, and whose passage through the mitochondrial internal membrane generates an electrical gradient that provides the necessary energy to form ATP or adenosine triphosphate. In addition, the malfunction of mitochondria can also raise the production of Reactive Oxygen Species.

Thus, essential biological functions, such as food metabolism, exercise and numerous pathologies, are closely linked to their production. Peroxisomes are also a source of Reactive Oxygen Species. They are extremely rich in oxidases and generate  $H_2O_2$ , purified by specific enzymes (catalases) and transformed into water (Browning and Horton, 2004).

Polymorphonuclear leukocytes are also an important source of Reactive Oxygen Species when activated by various proteins that act specifically on them, such as interleukins. This situation occurs particularly in inflammatory processes (Franzini et al., 1993).

Metals, such as cobalt, copper, chromium, iron and vanadium can make redox cycles where a single electron can be accepted or donated by the metal. This action catalyses reactions that can produce Reactive Oxygen Species (Ballester, 1996). Most enzymes that produce Reactive Oxygen Species contain one of these metals. The presence of these metals in biological systems without being in the form of a complex (i.e. not associated with a protein or other type of protection of the metal complex) can significantly increase the level of oxidative stress.

Certain organic compounds can also produce Reactive Oxygen Species. Oxidative stress produced by the reducing agent uric acid may be involved in stroke and metabolic syndrome (Lanaspa et al., 2012) (Gherghina et al., 2022). Likewise, the production of Reactive Oxygen Species in the presence of homocysteine in homocystinuria, as well as in arteriosclerosis and stroke (Guieu et al., 2022).

Reactive Oxygen Species also originate from environmental changes, such as exposure to ionizing radiation, X-gamma rays, ultraviolet light, atmospheric contaminants (ozone, nitrous oxide, carbon monoxide, sulphur dioxide, carbon tetrachloride, refineries, paper mills), combustion of organic compounds with smoke production (meats, cigarettes, industrial pollution), xenobiotics (pesticides, herbicides, fungicides) and some drugs (paracetamol).

Oxidative stress can damage the components of the cells (proteins, lipids and DNA), negatively affecting their functions (Monaghan et al., 2009). In lipids (unsaturated fatty acids), lipid-rich structures, such as cell membranes and lipoproteins, are damaged. In the former, permeability is altered, leading to oedema and cell death, and in the latter, oxidation of LDL, the genesis of atheromatous plaque.

With proteins, amino acids (phenylalanine, tyrosine, tryptophan, histidine and methionine) are preferably oxidised, and the result is cross-linking of peptide chains, fragmentation of the protein and formation of carbonyl groups, preventing the normal development of its functions (ionic transporters of membranes, cell receptors and messengers, enzymes that regulate cell metabolism, etc.).

Damage to the nucleic acids produces modified bases, with severe consequences on the development of mutations and carcinogenesis on the one hand, or loss of expression due to damage to the specific gene, on the other.

Oxidative stress participates in the primary etiopathogenic mechanisms of a large number of pathologies with high prevalence in our society, such as atherosclerosis, cancer, diabetes, neurodegenerative pathologies (Cui et al., 2004), such as Parkinson's disease (Reale et al., 2012) and Alzheimer's disease (Behl, 2005), or periodontitis (Sczeganik et al., 2020), clearly related to ageing (Olanow, 1993) (Pisoschi and Pop, 2015).



However, Reactive Oxygen Species may prove beneficial under certain circumstances. Thus, they can act as messengers in cell signalling, growth, cell differentiation, removal of infected or malignant cells and destruction of pathogenic organisms (Niki, 2014). However, they should be kept at adequate levels since their excessive production, as discussed above, can produce oxidative stress and cell damage (Venza et al., 2021) (Zahra et al., 2021).

#### **4. Antioxidants and their mechanism of action**

Foods that are a regular part of our diet contain various nutrients, some of which offer clear protection against oxidative stress. These include some vitamins, such as vitamin C, the various isomers of tocopherols and tocotrienols, vitamin A precursors, and some metals, such as copper, manganese, selenium and zinc.

Antioxidant substances can act at five different levels (Monaghan et al., 2009).

The first level consists of avoiding the univalent oxygen reduction by enzymatic systems capable of effecting consecutive quadrivalent reduction without releasing partially reduced intermediaries. This is achieved with great efficiency by the cytochrome oxidase system of the mitochondrial respiratory chain, responsible for more than 90 % of oxygen reduction in the human body.

The second level is constituted by enzymes specialised in capturing the superoxide anion radical ( $O_2^{\cdot-}$ ).

The third level refers to a group of enzymes specialised in neutralising hydrogen peroxide. Among them is catalase, found in peroxisomes.

In the fourth level, the hydroxyl radical can be neutralised by alpha-tocopherol or alpha-tocotrienol, the biologically active form of vitamin E, due to its hydrophobicity found in biological membranes where its protection is critical. Also, vitamin C or ascorbic acid, a reducing agent or electron donor that reacts quickly with the radical  $OH^{\cdot}$ .

Finally, once the molecular damage has occurred, there is a fifth level of defence that consists of repair of damaged molecules. It has been shown that Reactive Oxygen Species are capable of causing DNA chain breakdowns and inducing mutagenesis, but there are enzymatic repair mechanisms that allow the restoration of genetic information.

Antioxidants can be classified into two categories: enzymatic and non-enzymatic systems.

Among the antioxidant enzymatic systems, we find:

- Superoxide dismutase: an enzyme that catalyses the conversion of the superoxide anion to hydrogen peroxide. Several molecular forms have been disclosed: SOD-1, which acts in the cytosol and requires copper and zinc; SOD-2, which acts in the mitochondria and requires manganese; and SOD-3, which acts in the extracellular space and requires copper and zinc (Miao and St. Clair, 2009).
- Glutathione peroxidase: located in the cytosol and mitochondria, uses selenium as a cofactor. It catalyses the metabolism of hydrogen peroxide.
- Catalase: located in the peroxisomes, catalyses the metabolism of hydrogen peroxide.

The activity of these enzymes depends on the availability of oligoelements with antioxidant action (copper, manganese, selenium and zinc).

Among non-enzymatic antioxidant systems, we find certain molecules that react directly with Reactive Oxygen Species, avoiding damage to other molecules. The most relevant are vitamin E, located in the membrane, dehydroascorbic acid, glutathione, carotenes, uric acid, taurine and flavonoids.

When reacting with a free radical, the antioxidant yields an electron, oxidising and transforming itself into a weak non-toxic free radical and that, in some cases, can be regenerated to its primitive form by the action of other antioxidants, like vitamin E. Not all antioxidants act in this way, and the so-called enzymatic antioxidants catalyse or accelerate chemical reactions using substrates which in turn react with free radicals. Each antioxidant has an affinity to a certain free radical or to several.

However, these antioxidants can also have a prooxidant behaviour depending on various factors, such as the dose (amount ingested), the type of antioxidant and the matrix (food components) in which it is found. Thus, numerous studies show the beneficial effects of the use of antioxidants, whereas some others show the opposite effect (Martin and Appel, 2010) (NCCIH, 2013).

## **5. Metals with antioxidant/prooxidant effects**

Among the antioxidant metals with potential prooxidant effects, we can find copper, manganese, selenium and zinc, which are detailed below.

### **5.1 Copper**

#### **5.1.1 Biochemical functions**

Copper is an essential oligoelement that is part of the catalytically active protein centre of numerous metalloenzymes (cuproenzymes), which play a fundamental role in the oxide-reduction and electron transfer reactions. There are about 12 cuproproteins in humans that participate in a broad spectrum of functions, including cytochrome C oxidase, amine oxidases and zinc/copper-superoxide dismutase, whose activity is decisive in reducing the concentration of superoxide anions, as well as in neutralising the effects of Reactive Oxygen Species (ANSES, 2016) (Collins, 2021).

#### **5.1.2 Dietary Reference Values (DRV)**

EFSA provides Adequate Intake (AI) values, i.e. the estimated value when a Population Reference Intake (PRI) value cannot be established because an Average Requirement (AR) value could not be determined. An Adequate Intake (AI) is the level of average daily intake observed in an apparently healthy population group that is assumed to be adequate.

In the case of copper, EFSA provides values of Adequate Intakes (AI), defined based on the average intakes observed in several countries of the European Union because there is no evidence of copper deficiency in the European population (EFSA, 2015).

Likewise, EFSA establishes the maximum tolerable upper intake level (UL), i.e. the highest levels of daily intake at which it is likely that no adverse health effect is observed in the majority of individuals in the general population, taking into account all dietary sources (EFSA, 2006).

Both parameters are shown in Table 3.

**Table 3.** Copper Dietary Reference Values (DRV)

Population group	Age	Adequate Intake (AI) (mg/day) (EFSA, 2015)	Tolerable upper intake level (UL) (mg/day) (EFSA, 2006)
Infants	7-11 months	0.4	Not described
Children	1-2 years	0.7	1
	3 years	1	1
	4-6 years	1	2
	7-9 years	1	3
	10 years	1.3 (boys) 1.1 (girls)	3
	11-17 years	1.3 (boys) 1.1 (girls)	4
Adults	≥18 years	1.6 (men) 1.3 (women)	5
Pregnant women	≥18 years	1.5	Not described
Lactating women	≥18 years	1.5	Not described

In the non-exhaustive sampling performed on the 98 food supplements containing the words “antiox” or “detox” in their trade name that have been reported in the RGSEAA in 2019, 2020 and 2021, copper appears in 9.2 % of said supplements, with total daily doses ranging from 400 µg/day to 1.56 mg/day.

### 5.1.3 Copper deficiency

Copper deficiency is relatively rare. Given the wide range of enzymes that use copper as a cofactor, the symptoms of copper deficiency are diverse. These include:

- Hypochromic microcytic anaemia.
- Leukopenia.
- Neutropenia.
- Myelodysplasia.
- Hypercholesterolaemia.
- Skin and hair depigmentation.
- Defective elastin formation.
- Neurological symptoms, myelopathy, which can produce irreversible damage.
- Osteoporosis and scoliosis in children.

In our environment, copper deficiency is primarily observed in people with diseases or situations that produce malabsorption (e.g. celiac disease, digestive surgery, bariatric surgery) or in connection with an excessive intake of zinc in the form of supplements, which competes with the intestinal absorption of copper (Nishito and Kambe, 2018) (Altarelli et al., 2019).

### 5.1.4 Risk of toxicity

Copper is an essential trace metal that is required for the catalysis of several important cellular enzymes. However, since excess copper can also damage cells due to its potential to catalyse the generation of toxic Reactive Oxygen Species, copper transport and cellular copper content are strictly regulated. Under normal circumstances, homeostatic mechanisms prevent a copper overload from occurring in the event of excessive intake. Excess copper has been shown to cause health problems only under certain specific circumstances, such as Wilson's disease, which is caused by a mutation in ATP7B, a transport protein similar to ATP7A but expressed in different tissues. In this disease, the protein is defective in the liver and excretion is inhibited, which causes copper to initially accumulate in the liver, followed by the brain, heart, kidneys, and eyes. Liver injury progresses over time to produce cirrhosis with liver failure and neurological and psychiatric symptoms of varying severity (Czlonkowska et al., 2018).

Further, copper toxicity has been associated with neurodegenerative diseases, including Alzheimer's disease or Parkinson's disease (Mezzaroba et al., 2019) (Bisaglia and Bubacco, 2020) (Ejaz et al., 2020).

## 5.2 Manganese

### 5.2.1 Biochemical functions

Manganese is an essential metal and is the twelfth most abundant element on Earth. It can be found in a wide variety of salts and is therefore distributed in air, soils and water, resulting in its presence in legumes, rice and nuts, as well as in high amounts in unrefined cereals. It is also found in fish, seeds, chocolate, tea, and some fruits, such as pineapple and *açaí* (Horning et al., 2015).

Manganese is part of the group of divalent metals, Mn (II). However, we can also find in the organism Mn (III), which to avoid oxidative stress is reduced to Mn (II) by a ferrereductase to avoid an excess of oxidative stress (Chen et al., 2015).

Manganese is part of a multitude of enzymes, being particularly involved in the metabolism of carbohydrates (as a cofactor of enzymes, such as pyruvate carboxylase or phosphoenolpyruvate carboxykinase and intervening in the synthesis and secretion of insulin) and lipids (cholesterol synthesis), in the formation of cartilage and bone (glucosyltransferase enzyme), in healing (prolidase enzyme), in the synthesis of nitrogen monoxide (arginase) and in antioxidant protection (manganese-superoxide dismutase). In addition, it is known that manganese is involved in brain development and that its concentration is related to alterations in neuronal biology and the cognitive system (both excess and deficiency of manganese affect the nervous system) (Itokawa, 2004) (Aschner and Erikson, 2017) (Erikson and Ashner, 2019).

### 5.2.2 Dietary Reference Values (DRV)

Table 4 shows the values of Adequate Intakes (AI) for manganese (EFSA, 2013). According to EFSA, tolerable upper intake levels (UL) for manganese could not be established due to limited human data and the absence of a NOAEL (No Observed Adverse Effect Level) available for critical effects in animal studies (EFSA, 2006). However, the Institute of Medicine (US) Panel on Micronutrients

does establish a tolerable upper intake level (UL) of 11 mg/day of manganese obtained from food (IOM, 2001).

**Table 4.** Manganese Dietary Reference Values (DRV)

Population group	Age	Adequate Intake (AI) (mg/day) (EFSA, 2013)
Children	7-11 months	0.02-0.5
	1-3 years	0.5
	4-6 years	1
	7-10 years	1.5
	11-14 years	2
	15-17 years	3
Adults	≥18 years	3
Pregnant women	≥18 years	3
Lactating women	≥18 years	3

In the non-exhaustive sampling performed on the 98 food supplements containing the words “antiox” or “detox” in their trade name that have been reported in the RGSEAA in 2019, 2020 and 2021, manganese appears in 10.2 % of said supplements, with total daily doses ranging from 0.5 mg/day to 3 mg/day.

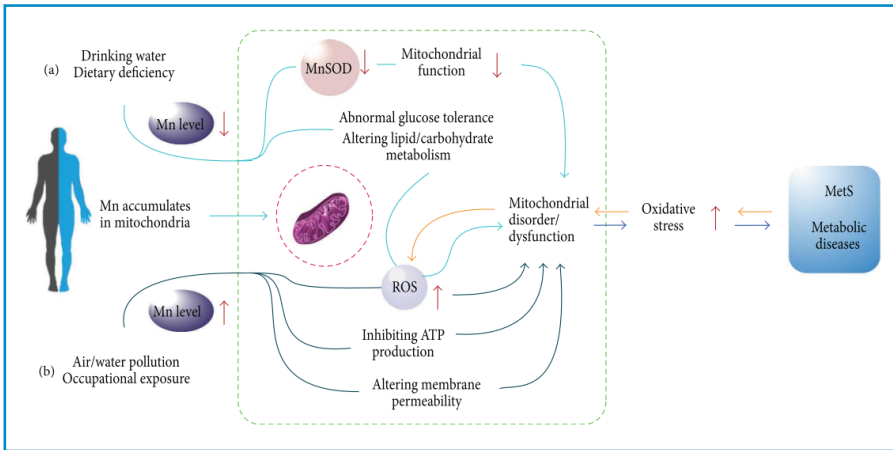
### 5.2.3 Intake deficit

Its deficiency in humans is not described as such (Horning et al., 2015).

### 5.2.4 Risk of toxicity

Manganese intake should provide a balance between the optimum point of its consumption, i.e. that necessary for the maintenance of its biological functions, and between excess, at which adverse effects occur when the established tolerable upper intake level (UL) is exceeded (ATSDR, 2012) (O’Neal and Zheng, 2015). Excessive intake causes different symptoms that are relatively similar, although not identical.

On the one hand, manganese seems to act by increasing oxidative stress, as seen in Figure 2. This excess manganese acts on the generation of Reactive Oxygen Species, which in turn increases oxidative stress in the mitochondria, increasing alterations in metabolic diseases, which seem to have a more significant impact on women than on men (Li and Yang, 2018).



(a) Manganese deficiency causes several adverse effects, such as impaired growth, poor bone formation and skeletal defects, reduced fertility and birth defects, as well as abnormal glucose tolerance and altered lipid and carbohydrate metabolism in both animals and humans. Thus, manganese deficiency can lead to mitochondrial dysfunctions through decreased levels of manganese-superoxide dismutase and altered metabolism of lipids and carbohydrates.

(b) Manganese overload may alter normal mitochondrial function by increasing mitochondrial Reactive Oxygen Species (ROS), inhibiting ATP production, and altering membrane permeability; in addition, it may cause mitochondrial dysfunction or disorder and, finally, cause metabolic syndrome (MetS) or metabolic diseases. An excess of ROS and oxidative stress would directly cause a metabolic syndrome (MetS) or metabolic diseases. If metabolic syndrome (MetS) or metabolic diseases occur, this will increase ROS production and oxidative stress and accelerate mitochondrial dysfunction or disorder.

**Figure 2.** Mechanisms of manganese in metabolic diseases through oxidative stress pathways. **Source:** (Li and Yang, 2018).

On the other hand, it seems that an excess of manganese could produce processes similar to Parkinson's disease, encompassed under the term "manganism", a process that is irreversible (Chen et al., 2015). Therefore, in food supplements an adequate dose of manganese must be ensured since, if the tolerable upper intake level (UL) is exceeded, it can lead to adverse situations in the body.

## 5.3 Selenium

### 5.3.1 Biochemical functions

Selenium is an essential metalloid for organic functioning, which is in animal tissues in the form of selenomethionine or selenocysteine. The vast majority of the functions of selenium are exercised by the intermediation of selenoproteins. In humans, the main selenoproteins identified are deiodinases, glutathione peroxidases, selenoprotein P and thioredoxin reductase. Deiodinases are involved in the metabolism of thyroid hormones, and the rest of the selenoproteins are enzymes that intervene in the defence against oxidative stress (ANSES, 2016).

### 5.3.2 Dietary Reference Values (DRV)

EFSA provides values of Adequate Intakes (AI) for this metal (EFSA, 2014a). In addition, EFSA sets certain tolerable upper intake levels (UL) (EFSA, 2006). Both parameters are shown in Table 5.

Population group	Age	Adequate Intake (AI) (µg/day) (EFSA, 2014a)	Tolerable upper intake level (UL) (µg/day) (EFSA, 2006)
Infants	7-11 months	15	Not described
Children	1-3 years	15	60
	4-6 years	20	90
	7-10 years	35	130
	11-14 years	55	200
	15-17 years	70	250
Adults	≥18 years	70	300
Pregnant women	≥18 years	70	300
Lactating women	≥18 years	85	300

In the non-exhaustive sampling performed on the 98 food supplements containing the words “antiox” or “detox” in their trade name and that have been reported in the RGSEAA in 2019, 2020 and 2021, selenium appears in 21.4 % of said supplements, with total daily doses ranging from 13.75 µg/day to 132 µg/day.

### 5.3.3 Intake deficit

According to AESAN estimates, the usual intake of selenium in the Spanish adult population is 86 µg/day, so, in general, there is no risk of insufficient intake. Only a limited number of women over 50 or 70 years may have insufficient selenium intakes.

### 5.3.4 Risk of toxicity

The excess of selenium, which can be caused both by its content in soils (Dinh et al., 2018), as well as by high intake of dietary supplements, comprises the excessive production of Reactive Selenium Species (RSeS), which induce alterations in DNA as well as being responsible for inhibiting the repair of damaged DNA. Thus, an excess of RSeS frequently affects the symptomatology associated with classic damage described for selenium excess: weakness, nausea, vomiting, diarrhoea and neurological symptoms like ataxia. Consumption of high-dose of selenium supplements induces a medical condition called selenosis, manifested by liver damage, hematopoietic disorders, infertility, nail damage and neurological disorders. The adverse effects also affect the endocrine system, altering the synthesis of thyroid hormones, selenium-dependent.

Selenium supplementation has an effect on the development of tumours and other pathologies, dependent on the dose and the characteristics of the population evaluated. Since selenium content

in food depends on the geographical location considered, studies conducted in a specific location cannot be extrapolated to other populations.

The dietary exposure levels above which selenium becomes toxic, and selenosis develops are difficult to establish because toxicity is affected by the selenium compounds present in the diet and, probably, by the combination of other dietary components and genotype interactions (Fairweather-Tait et al., 2011) (EFSA, 2014a).

## **5.4 Zinc**

### **5.4.1 Biochemical functions**

Zinc is an essential nutrient involved in numerous catalytic, structural and regulatory cellular functions. It participates in the activity of nearly 300 enzymes and in the various stages of protein synthesis, including the activation of enzymes involved in the nucleic acid synthesis, DNA repair, histone regulation and genome reading through transcription factors. Other functions include the stabilisation of the tertiary structure of certain peptide hormones and in the metabolism of polyunsaturated fatty acids and prostaglandins, as well as in the stabilisation of cell membranes (ANSES, 2016) (Lee, 2018).

In relation to oxide-reduction processes, zinc performs the following functions:

- It binds to sulfhydryl groups protecting them from modification by oxidants and reactive species, therefore, suppressing oxidative stress (Lee, 2018).
- It competes with iron and copper in their coordination environments and suppresses their redox activity, which can generate harmful hydroxyl radicals (OH<sup>•</sup>) through the Fenton reaction (Lee, 2005).
- It is a cofactor of the zinc/copper-superoxide dismutase enzyme (Lee, 2018).

Lee (2018) describes that both an excess and a deficiency of zinc lead to increased oxidative stress.

The entire biological dose-response relationship must be taken into account to interpret the effects of zinc in redox biology since prooxidant effects are observed when zinc concentrations are too low or too high (Lee, 2018). These concentrations oscillate in the organism in the picomolar range at the physiological level, in contrast to the cellular concentration, which is in the range of hundreds of micromolar. Therefore, given the concentrations in which zinc is found, it is not considered an oligoelement. The idea of its nature as a “trace” comes from the fact that only fairly small amounts (2-3 mg) are needed in the daily intake to maintain a total amount of 2-3 g in a 70 kg human.

### **5.4.2 Dietary Reference Values (DRV)**

Table 6 shows the Average Requirements (AR), Population Reference Intakes (PRI) and tolerable upper intake level (UL) indicated by EFSA (2006, 2014b).



**Table 6.** Zinc Dietary Reference Values (DRV)

Population group	Age	Average Requirement (AR) (mg/day) (EFSA, 2014b)	Population Reference Intake (PRI) (mg/day) (EFSA, 2014b)	Tolerable upper intake level (UL) (mg/day) (EFSA, 2006)
Infants	7-11 months	2.4	2.9	Not described
Children	1-3 years	3.6	4.3	7
	4-6 years	4.6	5.5	10
	7-10 years	6.2	7.4	13
	11-14 years	8.9	10.7	18
	15-17 years	11.8 (boys) 9.9 (girls)	14.2 (boys) 11.9 (girls)	22
Adults with Level of Phytate Intake (LPI) 300 mg/day	≥18 years	7.5 (men) 6.2 (women)	9.4 (men) 7.5 (women)	25
Adults with Level of Phytate Intake (LPI) 600 mg/day	≥18 years	9.3 (men) 7.6 (women)	11.7 (men) 9.3 (women)	25
Adults with Level of Phytate Intake (LPI) 900 mg/day	≥18 years	11 (men) 8.9 (women)	14 (men) 11 (women)	25
Adults with Level of Phytate Intake (LPI) 1200 mg/day	≥18 years	12.7 (men) 10.2 (women)	16.3 (men) 12.7 (women)	25
Pregnant women	≥18 years	(+) 1.3*	(+) 1.6*	25
Lactating women	≥18 years	(+) 2.4*	(+) 2.9*	25

\*These values should be added to AR and PRI for non-pregnant and non-lactating women.

In the non-exhaustive sampling performed on 98 food supplements containing the words “antiox” or “detox” in their trade name and that have been reported in the RGSEAA in 2019, 2020 and 2021, zinc appears in 22.5 % of said supplements, with total daily doses ranging from 2 mg/day to 20 mg/day.

#### 5.4.3 Intake deficit

The main food groups that contribute to zinc intake are meat and meat products, cereals and cereal products, milk and dairy products (EFSA, 2014b).

According to AESAN estimates, the usual intake of zinc in the Spanish adult population is 7.4 mg/day. In adults, more than 60 % of men and more than 40 % of women have an insufficient zinc intake.

Zinc deficiency affects more than 15 % of the world’s population (Ciubotariu et al., 2015). Zinc deficiencies have been described as a side effect of malabsorption problems, specifically in diets with very high fibre content, digestive pathology, gastrointestinal surgery, including bariatric surgery, alcoholism, including liver cirrhosis, kidney diseases and metabolic disorders (Lee, 2018). In the case of opioid users, an imbalance in the distribution of this metal has also been detected (Ciubotariu et al., 2015).

There are also studies that associate its deficiency with defects in the immune and neural systems (Lee, 2018). Symptoms of severe zinc deficiency include impaired growth and development,

delayed sexual maturation, rash, chronic diarrhoea, impaired wound healing and behaviour problems.

#### 5.4.4 Risk of toxicity

The 50 % lethal oral dose for zinc is approximately 3 g/kg body weight, which is 10 times that of cadmium and 50 times that of mercury (EFSA, 2014b) (Lee, 2018). It is very rare to reach these doses. However, three main routes of entry have been described by which zinc can reach this toxic level in the human body: through inhalation of zinc oxide in powder form, through the skin or by ingestion. Several studies have shown that exposure to high concentrations of zinc (up to millimolar) or industrial exposure does not cause severe health problems (Plum et al., 2010). Excessive oral intake of zinc (greater than 150 mg/day) for long periods may induce symptoms of copper deficiency (Lee, 2018). In addition, it has been shown that this metal suppresses anti-inflammatory responses, causing oxidative stress at the cellular level via suppression of metabolism and mitochondrial functions thanks to the increase of Reactive Oxygen Species, possibly causing cell death (Lee, 2018).

### 6. Sampling of food supplements with copper, manganese, selenium or zinc

A sampling of food supplements containing copper, manganese, selenium or zinc has been carried out in the RGSEAA database. The search criterion has been that the name of the mineral was present in the trade name and/or in the list of ingredients and that the products had been notified or modified recently (depending on the mineral, 2019-2022). With these criteria, 40 supplements have been identified for each of the minerals: copper, manganese, selenium and zinc.

Table 7 shows the percentage of the Nutrient Reference Value (NRV) that covers the highest daily amount recommended by the manufacturer for each one of the minerals. According to these data, the maximum content observed and its corresponding tolerable upper intake level (UL) for each mineral is: copper: 2.14 mg/day (UL: 5 mg/day); manganese: 4 mg/day (UL: 11 mg/day); selenium: 200 µg/day (UL: 300 µg/day) and zinc: 25 mg/day (UL: 25 mg/day).

**Table 7.** Comparison of the highest recommended daily amounts of copper, manganese, selenium and zinc in supplements identified in a food supplement sampling with the tolerable upper intake levels (UL)

	<b>Copper (mg/day)</b>	<b>Manganese (mg/day)</b>	<b>Selenium (µg/day)</b>	<b>Zinc (mg/day)</b>
Tolerable upper intake level (UL)	5 (EFSA, 2006)	11 (IOM, 2001)	300 (EFSA, 2006)	25 (EFSA, 2006)
Dietary Reference Value (DRV) in adult men and women	1.3-1.6 (AI) (EFSA, 2015)	3 (AI) (EFSA, 2013)	70 (AI) (EFSA, 2014a)	6.2-12.7 (AR) (EFSA, 2014b)
				7.5-16.3 (PRI) (EFSA, 2014b)

**Table 7.** Comparison of the highest recommended daily amounts of copper, manganese, selenium and zinc in supplements identified in a food supplement sampling with the tolerable upper intake levels (UL)

	<b>Copper (mg/day)</b>	<b>Manganese (mg/day)</b>	<b>Selenium (µg/day)</b>	<b>Zinc (mg/day)</b>
Usual adult intake	1.3 (Norway) (VKM, 2017)	4.2 (Sweden) 3.9 (Denmark) 2.7-3.1 (France) 3.3-3.6 (Ireland) (VKM, 2018)	86	7.4
Maximum percentage of the Nutrient Reference Value (NRV) of the labelling	214.2 %	200 %	364 %	250 %
Maximum amount in supplements calculated through the percentage of the NRV	2.14	4	200	25

AI: Adequate Intake; AR: Average Requirement; PRI: Population Reference Intake (97.8 %).

For copper, the usual intake in Norwegian adults is 1.3 mg/day, and the supplement with more copper involves taking 2.14 mg/day, so it would not exceed the tolerable upper intake level (UL).

For manganese, the intake (in other countries) generally covers the recommendation. Assuming an intake of 4.2 mg/day (Sweden) and a supplement of 4 mg/day (highest daily maximum amount), the tolerable upper intake level (UL) would not be reached.

For selenium, the usual intake in adults (86 µg/day) reaches the Adequate Intake (AI), although when adding the highest daily maximum amount of a supplement (200 µg/day) to said usual intake, the amount would be quite close to the tolerable upper intake level (UL) (95.33 % with respect to said limit).

In the case of zinc, with the usual intake in adults (7.4 mg/day), the recommendation may not be reached in every circumstance; however, considering only the highest maximum daily amount of one of the supplements (25 mg/day) it would reach the tolerable upper intake level (UL), and adding this to the usual intake, it would exceed this limit.

Therefore, and considering the 40 supplements sampled for each mineral, except for one supplement with zinc, adding the amount provided by the diet and that of the food supplements with the highest content of these minerals, the tolerable upper intake level (UL) would not be exceeded.

## Conclusions of the Scientific Committee

- Oxidative stress is one of the etiopathogenic mechanisms of a large number of pathologies with high prevalence in our society.
- The divalent metals copper, manganese, selenium and zinc have demonstrated to contribute to the protection of cells against oxidative stress by acting as cofactors of antioxidant enzyme systems. These minerals (metals) are present as ingredients in a large number of foods and food supplements, and have approved health claims related to the protection of cells against oxidative stress under the European regulatory framework.

- Scientific literature suggests that these metals could have prooxidant effects in certain doses or conditions. It is recommended that studies be carried out with an adequate methodology to evaluate these effects thoroughly and under which conditions they occur, as well as the adverse effects that could derive from them.
- The supplementation of copper, manganese, selenium and zinc through fortified foods and food supplements in the healthy adult population is safe under the conditions established by the European regulation.
- However, according to the scientific literature, food supplements with antioxidants have not demonstrated a decreased risk of developing cardiovascular disease or cancer.
- In addition, in the case of pregnant or lactating women, children, the elderly, and people with certain pathologies, supplements should only be consumed after consultation with a health-care professional since there is little information on the safety of many substances at these stages and, sometimes, they may have interactions with medicines. In no case should they replace the use of medicines without adequate medical supervision.
- Compounds that protect cells from oxidative stress are found naturally in many foods. Therefore, a varied and balanced diet is sufficient to meet the nutritional needs in terms of compounds with an antioxidant action in the healthy population. Only in the case of zinc, there is a certain deficit in Spanish adults, since in more than 60 % of men and in more than 40 % of women, the intake of zinc is insufficient.
- The maximum amounts of minerals that could be used in food supplements per daily intake recommended by the manufacturer, as well as the amount that could be added to foods should be established taking into account the following factors:
  - the maximum levels of safety of vitamins and minerals, as established through scientific risk assessment on the basis of recognised scientific data, considering, as appropriate, the different degrees of sensitivity of different categories of consumers;
  - the intake of vitamins and minerals from other food sources, as already provided for in Directive 2002/46/EC on food supplements (EU, 2002) and Regulation (EC) No. 1925/2006 (EU, 2006b) on the addition of vitamins and minerals and of certain other substances to foods.

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